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PATHOLOGY OF HEMORRHAGIC FEVER

A COMPARISON OF THE FINDINGS—1951 AND 1952 *

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Hemorrhagic fever first appeared in United States troops in Korea in 1951. Patients were treated in many hospitals, and methods of treatment varied considerably, but included the liberal use of intravenous fluids. The pathologic findings in 61 fatal cases were reported at the end of 1951,[1,2] and it was concluded that three features were prominent and characteristic: hemorrhage, particularly in the renal medulla, right atrium, and gastrointestinal submucosa; a peculiar type of necrosis of the renal medulla, anterior lobe of the pituitary body, adrenal gland, and, occasionally, islands of Langerhans; and a mononuclear cellular infiltration of the myocardium, pancreas, spleen, and liver. The abnormal physiologic processes were not entirely understood but shock caused many of the deaths during the early stages of the disease and thereafter renal failure was the important cause of death.

In 1952, a special center was established in Korea for the treatment of patients with hemorrhagic fever. They were brought to this hospital by helicopter to avoid the trauma and delay of road transportation. Emphasis was placed on fluid restriction to give a slightly negative balance. Concentration in one hospital of physicians, nurses, and corpsmen experienced in the treatment of this disease resulted in a considerable improvement in the general medical care. The laboratory facilities made available permitted close observation of electrolyte changes. In addition, highly trained investigators studied the physiologic changes and evaluated various forms of therapy. Fundamental concepts not previously clearly recognized were examined.

Possibly because of the changes in treatment, the frequency and severity of certain lesions differed in 1952 from those observed in

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1951. The present report attempts a comparison of these differences in fatal cases during the 2 years and presents new interpretations of clinicopathologic findings.

Materials and Methods

The 406th Medical General Laboratory in Tokyo received necropsy material from 71 patients dying of hemorrhagic fever in 1951, and from 50 in 1952. Six* of the 1951 cases had insufficient clinical information and were dropped from the study. This report is, therefore, based on 115 fatal cases of hemorrhagic fever. The pathologic changes observed in 39 patients who died at the Hemorrhagic Fever Center in 1952 have recently been described.[3]

In 1952, as in 1951, most of the fatalities occurred in Korea and the necropsies were performed at the Hemorrhagic Fever Center. Stained sections and fixed tissues were studied at the First Medical Field Laboratory in Korea and were reviewed at the 406th Medical General Laboratory. Protocols were much more complete in 1952 than in 1951, permitting more accurate clinicopathologic correlation. The same general procedures and special staining techniques used in 1951,[1,2] plus the Schiff periodic acid and the Feulgen stains, were applied as indicated to the 1952 material.

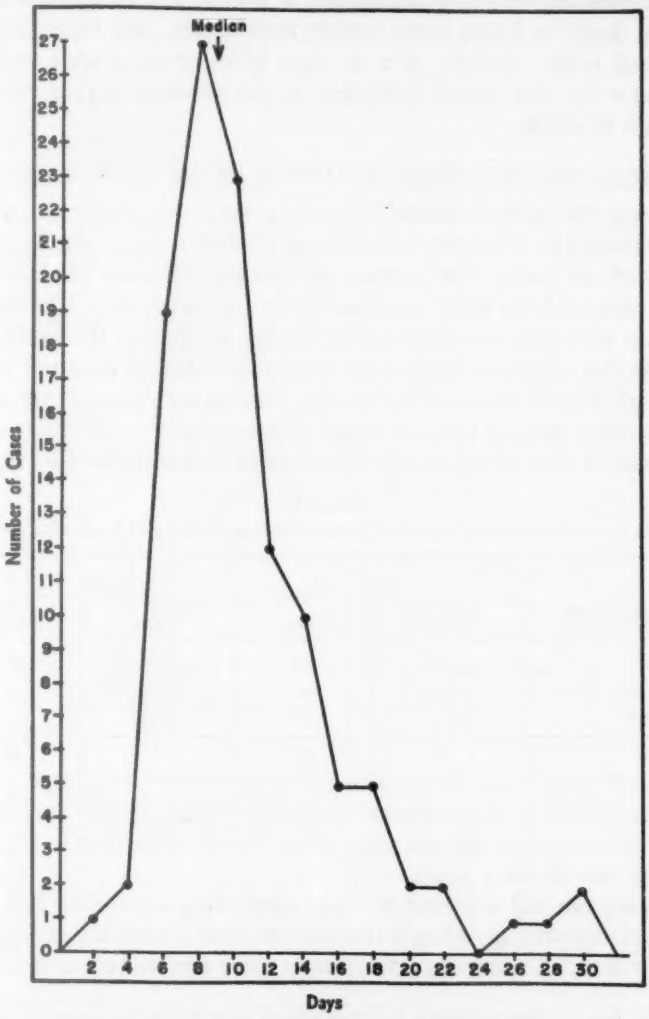
In the 1951 reports[1,2] the significance of the different clinical stages was not fully appreciated. Correlation of cause of death with variations in organ weights and histologic changes was based either on an arbitrary duration of disease (7-day intervals) or not related to the duration of disease at all. For purposes of the present report, all necropsy protocols and histologic sections from both years were reviewed in the order received and evaluated according to the clinical stage of the disease at the time of death. The sections of heart and kidney were then re-studied in the new order.

Basis for Clinicopathologic Correlations

One of the significant concepts resulting from the study of hemorrhagic fever in 1952 was the division of the disease into fairly well defined stages of fever, hypotension or shock, oliguria, diuresis, and recovery.[4-6] The signs and symptoms during the febrile and hypotensive stages appeared to be due to a circulating toxic agent and consisted of high fever, circulatory collapse, a leukemoid blood reaction, and various hemorrhagic phenomena. Patients dying at this time had severe shock which did not respond to therapy. Death during the oliguric and diuretic stages resulted from renal failure, pulmonary

* Duration of disease was: unknown, 4, 7, 8, 8, and 11 days, respectively.

edema, pulmonary suppuration, cerebral hemorrhage, or a combination of these. The relation between the early hypotension and the late acute renal failure was not apparent since patients showing little or no evidence of shock nevertheless developed renal involvement.[7] So



Text-fig. 1. Duration of disease for 111 fatal cases, 1951 and 1952.

uniform was the renal involvement that urinary changes were required to confirm the clinical diagnosis of hemorrhagic fever, and typical kidney changes were found in all fatal cases at necropsy.

The following criteria were used in determining the clinical stage of the disease at the time of death: hypotensive, if spontaneous return of the blood pressure to normal or to hypertensive levels had not occurred; oliguric, if, in addition to decreased urinary output, the blood pressure had been maintained at normal or hypertensive levels for at least 24 hours even though hypotension had recurred as a terminal event; diuretic, if 2 or more liters of urine were excreted during a 24-hour period regardless of the subsequent recurrence of oliguria or shock.

Data for Comparison of Disease in 1951 and in 1952

It was the general clinical impression that hemorrhagic fever had not changed in character, severity, or clinical findings during the 2 years of the study. The average and median duration of disease in fatal cases did not differ significantly in 1951 and 1952. The largest number of deaths occurred on the 8th day of disease, the median on the 9th day of disease, and 76 per cent of the fatalities occurred before the 13th day of disease (Text-fig. 1). During both years of the study the average interval between onset of disease and hospitalization for all patients who died was 3.6 days. When broken down by stage of disease (Table I), it was evident that delay in hospitalization after the onset of symptoms was not responsible for any difference in the case fatality rate in the 2 years.

Table I

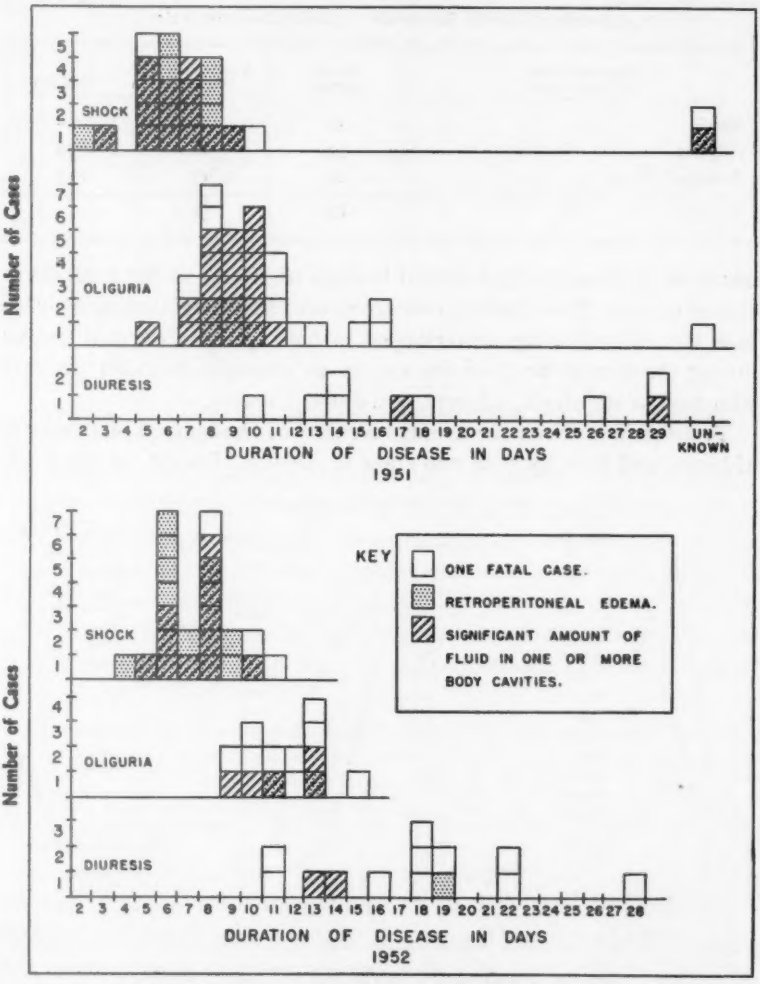
Available Information on Interval Between Onset of Disease and Hospitalization

Stage of disease at time of death	1951		1952	
	Average no. of days	Deaths	Average no. of days	Deaths
Shock	3.15	20	3.50	22
Oliguric	3.92	26	4.0	13
Diuretic	4.12	8	3.45	11
Totals		54		46

During the fall epidemic of 1951 (beginning September 12), 876 cases of hemorrhagic fever were reported with a case fatality rate of 6.4 per cent.* During 1952, there were 1,067 reported cases of hemor-

* The first American experience with hemorrhagic fever during the spring and summer of 1951 was associated with a high case fatality rate. In retrospect, it is believed that this resulted from inadequate knowledge of the disease and non-diagnosis of many mild cases. Subsequently, beginning with the fall epidemic of 1951, medical officers were on the alert and suspected the presence of the disease in all patients showing unusual symptoms. For this reason, the comparison of case fatality rates is based on the reports for the second half of 1951 rather than on the reports for the entire year.

rhagic fever, with a case fatality rate of 4.9 per cent.[8] A difference in the number of deaths in the oliguric phase between the 2 years became evident when the expected mortality rate by stage of disease based on the 1951 fall epidemic experience was applied to the 1952 experience (Table II). Since it is the current belief that restriction of fluids de-



Text-fig. 2. Fluid retention and stage of disease at time of death by year and duration.

creases the fatality rate in acute renal failure, it appeared that the fluid restriction program instituted in 1952 decreased the case fatality rate in the oliguric stage of hemorrhagic fever.

Gross and Microscopic Changes at Necropsy

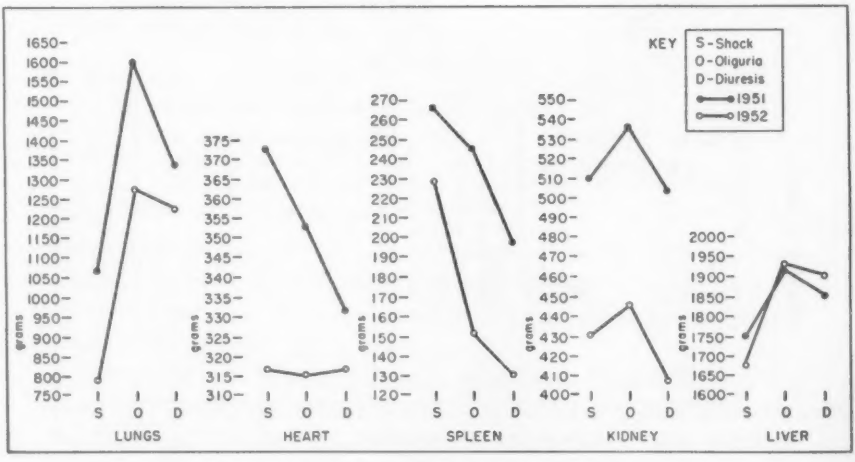
When fatal cases were studied by clinical stages of disease, it was realized that the duration of the different stages varied considerably among patients. Therefore, study of cases by arbitrary duration periods masked those changes related to stage of disease rather than duration of disease. Text-figure 2, concerned with fluid retention, also shows how the different stages overlapped in both years. Study of deaths during the second week of disease, as an example, included patients who died in the shock, oliguric, and diuretic stages.

Table II

Number of Deaths Expected in 1952 Based on 1951 Rates

Stage of disease at time of death	Number expected	Number which occurred	Variation
Shock	25	23	− 2
Oliguric	33	14	−19
Diuretic	10	13	+ 3
Totals	68	50	

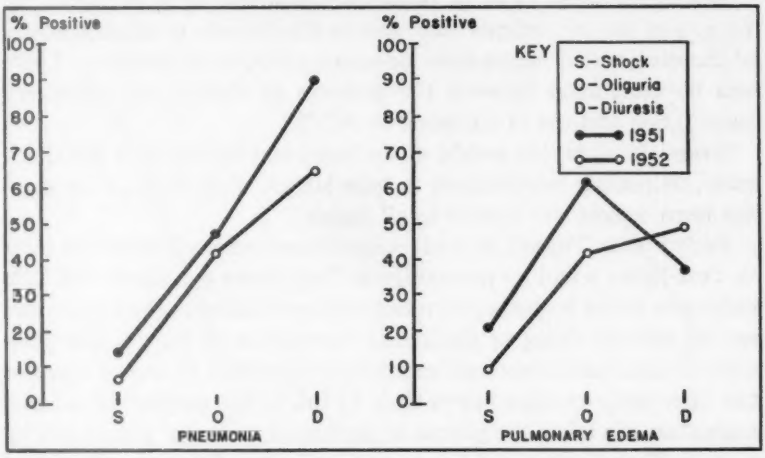
Text-figure 3 shows the average weights of the lungs, heart, spleen, kidneys, and liver by year and stage of disease. Except for the liver,



Text-fig. 3. Weights of organs by year and stage of disease.

these organs were consistently heavier in 1951 than in 1952 when compared by stage of disease and, with the exception of the heart, the weights of these organs increased or decreased in both years almost in the same proportion in the different stages of disease. The average

of the available estimated body weights of 46 patients in 1951 was 160¼ lbs. and of 46 patients in 1952 was 153 lbs., indicating that differences in organ weights probably were not due to differences in



Text-fig. 4. Presence of pneumonia and pulmonary edema (microscopic examination) by year and stage of disease.

the weights of the patients. In each year, 7 patients were Orientals.

Lungs. The increase in the average weight of the lungs in the oliguric and diuretic stages was due to the occurrence of pulmonary edema and pneumonia. The presence of either or both was recorded when the lung sections were studied microscopically (Text-fig. 4). In the diuretic stage, the effect of the decreased incidence of pulmonary edema on the weight of the lungs was not entirely balanced by the increased

TABLE III

Average Severity of Pneumonia as Seen Histologically by Year and Stage of Disease

	Shock		Oliguria		Diuresis	
	1951	1952	1951	1952	1951	1952
Number of patients	16	16	25	9	9	10
Average severity of pneumonia	0.33	0.17	0.95	1.82	1.89	2.27

incidence of pneumonia. Pneumonia occurred less frequently in 1952, but, when present, it was more severe than in 1951 (Table III). The severity of pneumonia was graded histologically* on a 1 to 4 basis,

* Histologic grading of the various tissues was accomplished by judging the degree of involvement in tissue sections from each patient in the group and dividing the sum of the grades for each group by the number of patients in each group. The factor resulting from this manipulation was considered the average severity of involvement.

grade 1 being small foci of bronchopneumonia, grade 3 being diffuse consolidation involving almost the entire section, and grade 4 being diffuse consolidation with suppuration.

During 1951, no cases of pulmonary suppuration were found. In 1952, 9 of the 27 patients who died in the diuretic or oliguric stages of the disease had suppurative pulmonary lesions at necropsy. There was no correlation between the presence or absence of pulmonary suppuration and use of cortisone or ACTH.

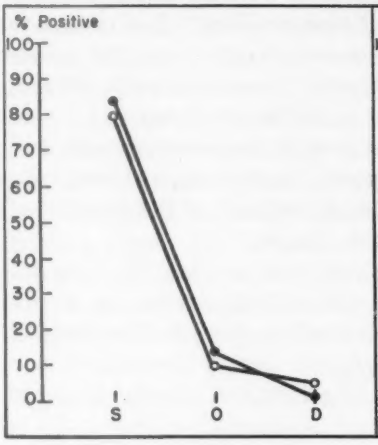
Heart. In 1951, the weight of the heart was increased in the shock stage, decreasing progressively in later stages, while in 1952 the average heart weight was normal in all stages.

Body Fluids. The presence of retroperitoneal edema is shown by cases in Text-figure 2 and by percentage in Text-figure 5a. There was little difference in the frequency of retroperitoneal edema in the 2 years and not all patients dying in shock had retroperitoneal edema. The presence of significant abnormal amounts of free fluid in one or more of the three body cavities (more than 25 ml. in the pericardial sac and more than 100 ml. in the pleural or peritoneal spaces) is also shown by cases in Text-figure 2 and by percentage in Text-figure 5b. Free fluid in body cavities was found more frequently in 1951 than in 1952 and in both years persisted in the oliguric and diuretic stages. Infrequently, pleural effusion was due to pneumonia.

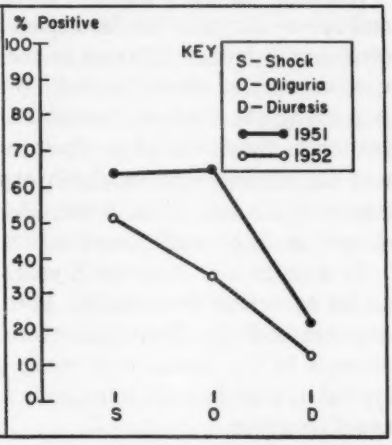
Microscopic Examination

Heart. When the cardiac sections were reviewed, it was realized that certain mild changes described in 1951 were present so frequently and were so unusual as to be almost diagnostic. In addition to the "hemorrhage" observed in all cases in the right atrium, there was a mild infiltration of cells into an edematous layer immediately beneath the intact and unaltered endothelium of the endocardium (Figs. 1 and 2). The infiltrate consisted principally of various mononuclear cells, including plasma cells and Anitschkow's myocytes, occasionally mast cells and eosinophils and, rarely, polymorphonuclear leukocytes. Such infiltrates, generally only a few cell layers thick, were found in all chambers, on occasion in the valves, and could be traced from the endocardium between muscle bundles into the interstitial tissue. Similar infiltrates frequently were found about small vessels, but nowhere were they consistently associated with an endothelial or intravascular reaction. Infiltrates, consisting of collections of a few mononuclear cells, were sometimes found beneath the endothelium of the aorta and coronary arteries. Special stains have failed to reveal inclusion bodies or rickettsia in these cells. There was little variation in the incidence

of these findings between 1951 and 1952, but in both years these changes were progressively less prominent and less frequent after the shock stage (Table IV). Some of the myocardial infiltrate found in



Text-fig. 5a. Presence of retroperitoneal edema by year and stage of disease.



Text-fig. 5b. Presence of free fluid in body cavities by year and stage of disease.

patients dying in the oliguric and diuretic stages may have been related to hyperkalemia, azotemia, and/or pulmonary infection.

In the edematous subendothelial layer of the right atrium the red blood cells frequently were massed so that cell outlines could not be

TABLE IV

Percentage of Cases Showing Cardiac Changes by Stage of Disease, 1951 and 1952 Combined

Histologic findings	Shock (39 cases)	Oliguria (32 cases)	Diuresis (17 cases)
	%	%	%
Subendocardial edema	100	82	53
Subendocardial infiltrate	97	78	65
Myocardial infiltrate	64	63	41

distinguished, while in the atrial myocardium pooling of blood was not frequent, the erythrocytes usually being diffusely distributed, almost in a mosaic pattern. Even in the cases of longest duration, no cellular reaction to the extravasated blood was found, iron stains were negative, and pigment-bearing phagocytes could not be found in either the myocardium or the epicardial fat. In the heart and in other areas of hemorrhage, considerable variation in the size of erythrocytes was observed. Extremely small red blood cells often were seen. Monroe

and Strauss[9] described similar changes, believed they resulted from fragmentation of larger cells without loss of pigment, and interpreted this as evidence of mechanical red blood cell destruction.

Kidney. Although necrotic renal lesions were much less frequent and severe in 1952, the fundamental changes described in 1951 were consistently found. Changes in the cortex generally were not prominent in cases of short duration. In cases of longer duration, tubules, principally distal convoluted tubules, tended to be dilated and to contain casts consisting of precipitated protein, desquamated cells, and material resembling hemoglobin pigment. Such sections showed mild edema of the interstitial tissue. A rough estimate of the duration of disease could be made, based on these changes.

In 2 cases, one from each year, there was necrosis involving the cortex as well as the medulla. Both were of long duration, 22 and 26 days respectively. Both had severe medullary necrosis. The areas of necrosis in the cortex were wedge-shaped, sharply demarcated, and typical of anemic renal infarcts, but no evidence of vascular occlusion could be found.

When the corticomedullary "hemorrhage" was re-examined, it was realized that hemorrhage resulting from rupture or loss of continuity of a vessel was rarely, if ever, present. In cases of short duration (5 days or less), engorged, dilated, but still recognizable vascular channels were seen. Thereafter, in many areas, although distinct vascular channels were not found and tubules were separated by fields of red blood cells (Fig. 3), special stains showed very delicate fibers lying among the red blood cells, suggesting an extremely dilated sinusoidal pattern. In some patients, including many dying in the diuretic phase, there was less congestion. In such cases, the interstitial tissue of the corticomedullary area was edematous and contained separated red blood cells.

Even in cases of long duration, there was little, if any, evidence of red blood cell destruction, although it appeared that hemorrhage was present very early in the disease and in most patients. It is now believed that these hemorrhages represent vascular engorgement and diapedesis of red blood cells rather than hemorrhage from ruptured vessels and that the lack of evidence of red blood cell destruction is related to the mechanism which produces these changes. Obstruction to the venous return by occlusion of renal vessels, the usual cause of these changes, has not been demonstrated. A few sections did show an infiltration of mononuclear cells in the walls of renal veins; in three sections, veins in the peripelvic fat showed thrombophlebitis. An attempt was made to tabulate vascular infiltrative changes but this was

not reliable because lesions could sometimes be found in cases from which a large number of kidney sections were available and not in those with but a few sections or in those not containing adequate portions of renal medulla.

In most cases, there was clearly recognizable tubular necrosis in the medulla (Fig. 4). When the involvement was more severe, groups of ghost-like tubules were seen lying sometimes in an area of congestion, but more frequently in an area of edematous acellular stroma. Such areas resembled anemic infarcts and sometimes were so extensive as to involve the entire pyramid (Fig. 5). There was a gradual transition from involvement of individual tubules to areas of definitely recognizable necrosis. For the purpose of this report, the criteria for focal necrosis were made more strict and only definitely recognizable necrotic areas involving interstitial tissue as well as tubules were tabulated. Table V shows both the incidence and severity of this lesion by

TABLE V

Incidence and Average Severity of Medullary Necrosis by Year and Stage of Disease

	Shock		Oliguria		Diuresis	
	1951	1952	1951	1952	1951	1952
Number of cases	20	23	27	14	9	13
Necrosis present	25%	4%	52%	36%	67%	23%
Average severity of necrosis	0.45	0.04	1.48	0.71	2.11	0.62

year and stage of disease, as graded in the histologic sections on a 1 to 4 basis. It is apparent that there was appreciably less focal necrosis in the medulla in the 1952 cases than in the 1951 cases.

No correlation could be found between the presence or absence of medullary necrosis and shock, retroperitoneal edema, fluid in body cavities, pneumonia, the use of vasopressor drugs, or the use of ACTH or cortisone. Some patients with little hypotension throughout the course of their disease had medullary necrosis while others did not. Some patients who survived severe shock, only to die in a later stage, had necrosis, while others did not. Necrosis appeared to be related to duration of disease but there were exceptions to this. In 1951 necrosis appeared in some cases of short duration (6 days) and was absent (17 days) or mild (29 days) in cases of long duration. In 1952, necrosis was not seen in cases of less than 10 days' or more than 22 days' duration.

Pituitary Body. The presence and severity of foci of necrosis in the anterior lobe of the pituitary body appeared related to the stage of disease at the time of death (Table VI). Approximately 58 per cent

of patients who died in the shock stage had pituitary necrosis and this was generally mild. All patients who died in the oliguric stage had pituitary necrosis which was frequently severe. In the diuretic stage, 17 of 18 patients had some degree of necrosis, but often, especially in the 1952 cases, this was so slight as to involve only a few cells. Table VI shows the degree of involvement based on a 1 plus to 4 plus scale

TABLE VI

Necrosis of Anterior Lobe of Pituitary Body by Year and Stage of Disease

	Shock		Oliguria		Diuresis	
	1951	1952	1951	1952	1951	1952
Number of cases	17	19	21	9	6	12
Necrosis present	53%	63%	100%	100%	100%	92%
Average severity of necrosis	1.2	1.0	3.1	2.9	2.5	1.9

as seen in histologic sections. Despite this evidence of frequent involvement of the pituitary body, it has not been demonstrated that pituitary insufficiency plays an important rôle in this disease.

Adrenal Gland. Table VII presents in a similar manner the data dealing with adrenal necrosis. The degree of histologic involvement was based on a scale of 1 to 4, with grade 4 representing necrosis of

TABLE VII

Necrosis of Adrenal Gland by Year and Stage of Disease

	Shock		Oliguria		Diuresis	
	1951	1952	1951	1952	1951	1952
Number of cases	20	22	29	13	8	13
Necrosis present	15%	23%	35%	0%	0%	15%
Average severity of necrosis	0.5	0.6	1.1	0	0	0.4

half an adrenal section, the most severe degree seen. Comparison of the 2 years showed a considerable difference in the degree and frequency of adrenal necrosis in the oliguric and diuretic stages.

Liver. Midzonal necrosis was noted in 24 cases with only 12 being of more than minimal degree. No correlation could be determined for stage of disease and there was an equal number each year.

Gastrointestinal Tract. In the esophagus, a mononuclear infiltrate of varying degree, associated in some cases with actual ulceration, was seen in most sections. These, however, were too few to permit study by stage of disease or by year.

Very little difference could be noted in gastrointestinal congestion when examined by year and stage of disease. It was frequently pres-

ent and often intense in all stages, but it was limited to the most superficial portion of the mucosa. In a few early cases, there was congestion at the base of the mucosa with edema of the area usually congested in later stages.

Discussion

There were two major differences in the treatment of patients in 1952, as compared to 1951. First was the better general medical care resulting from the concentration of patients in one hospital with treatment by physicians, nurses, and corpsmen experienced with the disease. Second was the emphasis placed on fluid restriction. The decrease in the average weight of organs and free fluid in body cavities in the 1952 series as compared to the 1951 series (Text-figs. 2 and 5b) was at least in part due to the fluid restriction program. Since the major decrease in mortality in 1952 occurred in the oliguric stage (Table II), it appeared that when hemorrhagic fever was treated as acute renal failure, some patients were saved and others were carried to the diuretic stage before succumbing.

Characteristic renal and cardiac changes were observed in necropsies of patients who had been ill for only a short time before death, indicating that the tissue damaging agent was active during the first few days of the disease. Clinical studies showed that the renal damage occurred early and in some ways resembled so-called lower nephron nephrosis which often follows a renal insult of short duration. Because the cellular reaction in the heart tended to be less intense when the disease was of long duration and because the hemorrhages and the infarct-like necrotic foci in the various organs of the same patient appeared to be of the same age, it was suspected that the agent causing tissue damage was active only during the first few days of the disease and that the processes seen in patients with disease of longer duration represented tissue reaction to previous injury. This concept was supported by the Russian reports[10] that the etiologic agent of hemorrhagic fever could be transmitted by blood and urine obtained only from patients who had been ill for less than 6 days.

The diversity of organs involved, the widespread vascular reaction, the cellular changes in the heart, and the peculiar type of hemorrhage in many organs suggested the presence of a circulating vascular toxin. Since, in most non-fatal cases, recovery was complete by all clinical criteria, it was concluded that the vascular changes were reversible and that hemorrhages were resorbed. One patient who died of acute hepatitis 3 months after recovery from a typical moderately severe attack of hemorrhagic fever had no evidence of damage resulting from the latter disease.

Because the foci of necrosis in the kidneys and pituitary and adrenal glands resembled infarcts and were usually associated with areas of hemorrhage, vascular dysfunction and anoxia were considered to be the more likely cause for focal necrosis than the direct action of a toxin. The cause of hemorrhage apparently was not the cause of necrosis since necrosis was never found in the right atrium. The changes in the renal tubules likewise appeared secondary to vascular changes and resultant local anoxia. Although the renal lesion of hemorrhagic fever might, therefore, be considered a form of so-called lower nephron nephrosis,[11] the hemorrhage at the corticomedullary junction in hemorrhagic fever was much more severe than the congestion in the same area in lower nephron nephrosis; infarct-like areas of necrosis have not been described in lower nephron nephrosis; and the focal interstitial infiltrates and granulomas found in that syndrome have not been observed in hemorrhagic fever.

The pituitary and adrenal changes did not appear to be an essential factor in the pathogenesis of the disease because they were not always present, patients showed no consistent response to ACTH and/or cortisone, and no case of pituitary cachexia has been reported among patients who recovered from the disease.

Moderate to severe retroperitoneal edema was present at necropsy in approximately 75 per cent of patients who died in the hypotensive phase of the disease. The remaining 25 per cent had little or no retroperitoneal edema. Fluid in other areas such as periorbital and subcutaneous tissues and large muscle groups, and free fluid in body cavities were not always present. It therefore appeared that, in some patients, escape of fluid from the vascular compartment was not essential for the development of the hypotensive phase. However, it was evident that in the majority of patients such fluid loss was frequent, sometimes massive, and, if not the primary cause of shock, contributed to and increased its effect on organ function.[12]

Retroperitoneal edema was rarely found in patients dying in the oliguric and diuretic stages although free fluid was often present in the body cavities. Clinical studies[13-16] showed that hemoconcentration and decreased circulating blood volume were present in many of these patients during the hypotensive phase and were quickly reversed with recovery from hypotension. It was, therefore, suspected that these patients had had retroperitoneal edema during the hypotensive phase and that the fluid had been rapidly resorbed. Because a few patients who died after recovery from shock showed retroperitoneal edema at necropsy and because a few patients had a low circulating blood vol-

ume in the post-hypotensive phase, it appeared that the resorption of retroperitoneal fluid was not necessary for recovery from the hypotensive phase.

Although previously present, the signs and symptoms of progressive renal failure did not become prominent until the patient recovered from the hypotensive phase of the disease. During the oliguric and diuretic phases some patients died because of renal failure with or without detectable electrolyte abnormality, some died of complications such as hemorrhage, pneumonia, and pulmonary abscess, and some died of severe acute pulmonary edema. The pneumonia showed no unusual features except for the large number with suppuration in 1952, apparently due to aspiration. In both years, but particularly in 1951, pulmonary edema was a frequent terminal event and appeared to be caused by the rapid return of large amounts of fluid to the vascular compartment rather than to cardiac failure.[12]

Summary

A comparative study has been made of the pathologic findings in patients dying of hemorrhagic fever in 1951 and 1952. There is evidence that the changes were similar in both years, but of a different degree of severity. It is suggested that these differences were related to differences in therapy rather than to a change in the character of the disease itself. It is suspected that fluid restriction and treatment as for acute renal failure were the important therapeutic changes and that a decrease in the number of deaths in the oliguric stage resulted from this approach.

It is believed that a toxin is elaborated and is active only during the early course of the disease. During this time there is evidence of widespread capillary and endothelial damage and apparently all subsequent manifestations of this disease are the result of this damage.

Re-evaluation of cardiac lesions indicates that an almost pathognomonic picture is present, especially in cases of short duration. The important feature is a mild subendothelial mononuclear cell infiltration.

Re-evaluation of the renal lesions indicates that hemorrhage due to loss of continuity of blood vessels was not present. It is believed that the so-called hemorrhage represents extreme congestion and dilatation of corticomedullary vessels with diapedesis of red blood cells. Diapedesis of red blood cells is also suggested as the mechanism of the hemorrhage in the right atrium.

Re-examination of the foci of necrosis in the kidneys and the pitui-

tary and adrenal glands suggests that these were the result of anoxic changes rather than due to the direct action of the etiologic agent or a toxin.

The recognized and controversial fluid balance changes were discussed. On the basis of the necropsy studies it is believed that in fatal cases these are severe, important, but secondary changes. Until the etiologic agent and a specific therapy are discovered, they will remain as significant problems in the management of this disease.

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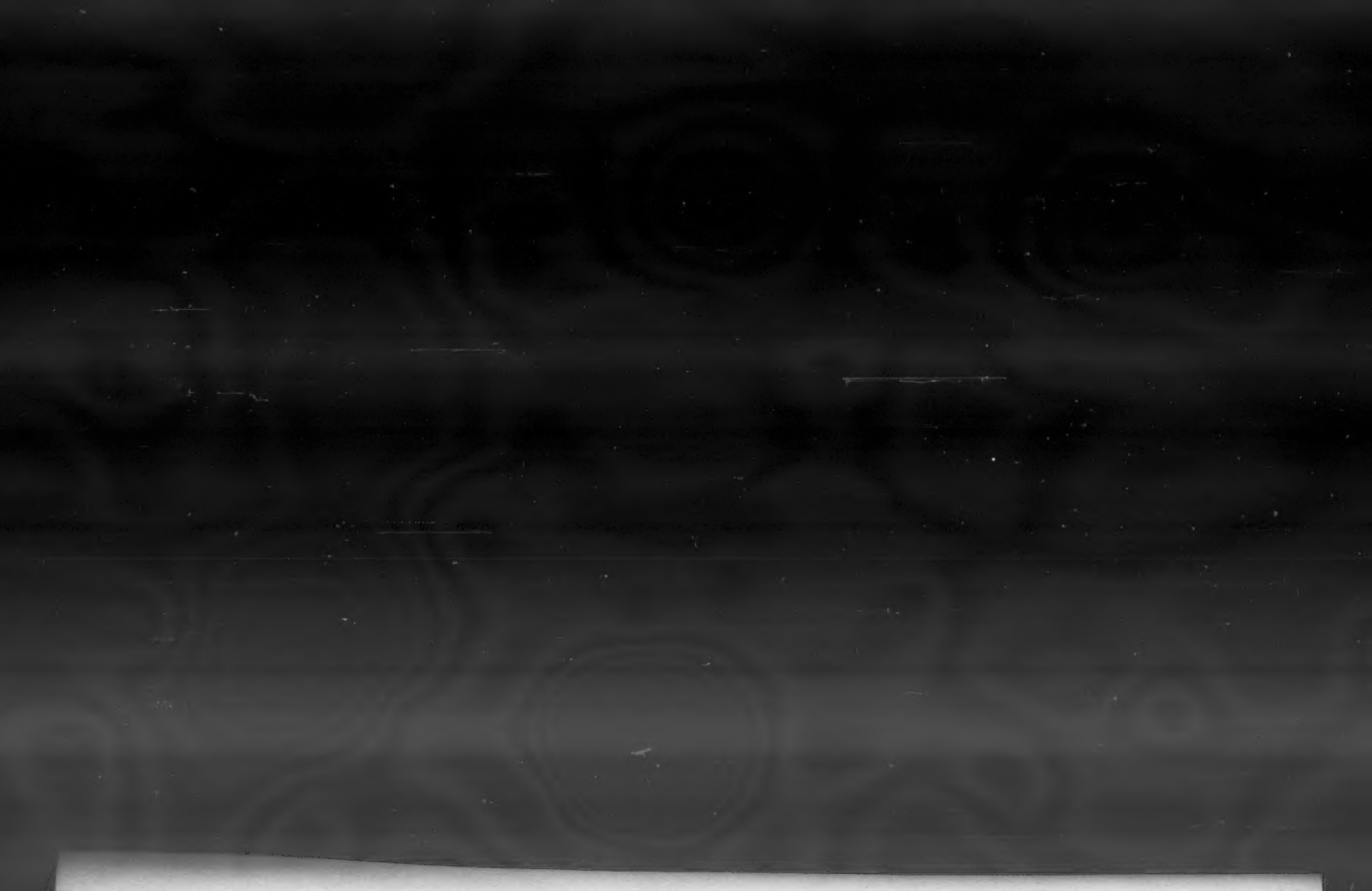
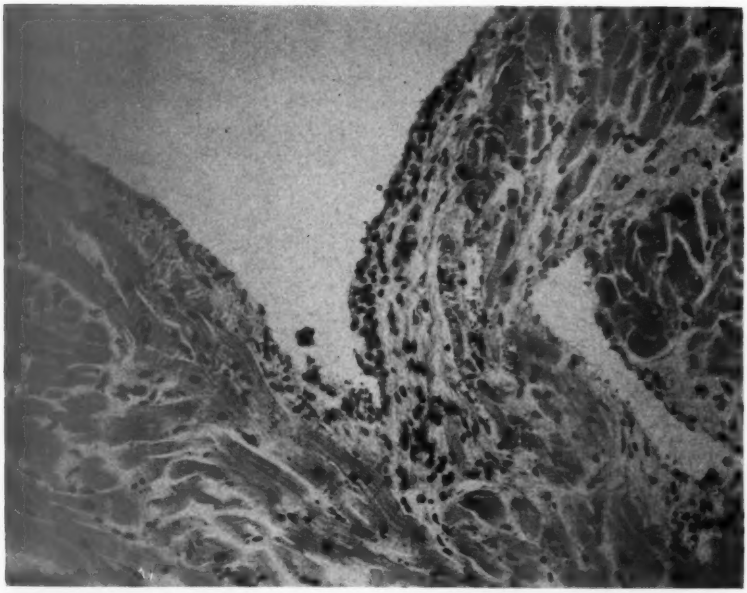
[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. Section from right ventricle showing subendocardial infiltrate and edema, mild myocarditis, and mild infiltrate beneath endothelium of a vein. Death on seventh day of disease in shock stage. Hematoxylin and eosin stain. × 160. (Armed Forces Institute of Pathology negative 53–23475.)

FIG. 2. Another field from the same section as that from which Figure 1 was taken, showing in greater detail the infiltrate and edema beneath the intact endothelium. Hematoxylin and eosin stain. × 555. (A.F.I.P. negative 53–23476.)





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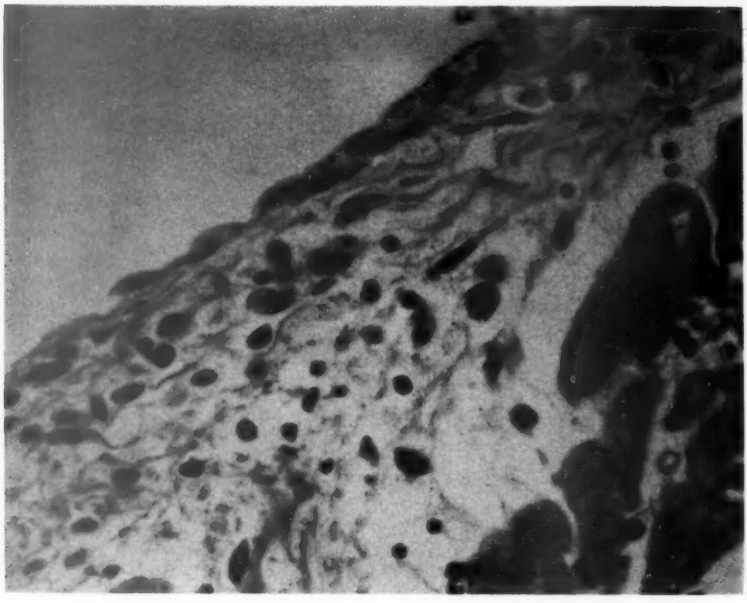

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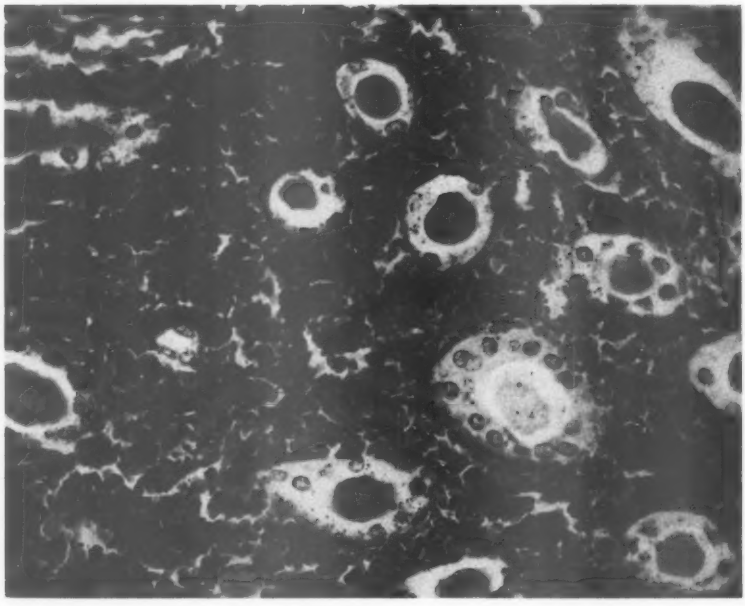
FIG. 3. Section from kidney at the corticomedullary junction, showing extreme engorgement, tubular casts, and tubular epithelial changes. Death on eleventh day of disease in shock stage. Hematoxylin and eosin stain. × 395. (A.F.I.P. negative 53–23489.)

FIG. 4. Section from a kidney at the corticomedullary junction showing congestion, tubular necrosis, and casts. Death on eighth day of disease in shock stage. Hematoxylin and eosin stain. × 400. (A.F.I.P. negative 53–23488.)

FIG. 5. Section from kidney at the corticomedullary junction and medulla showing congestion and large infarct-like area of necrosis. Death on 26th day of disease in diuretic stage. Hematoxylin and eosin stain. × 48. (A.F.I.P. negative 53–23490.)



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5

SIGNET-RING CELL CARCINOMA OF THE URINARY BLADDER*

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Adenocarcinomas of the urinary bladder are rare tumors. Wheeler and Hill,[1] in a review of the English literature, encountered 14 primary adenocarcinomas arising in normally placed urinary bladders, 12 in exstrophic bladders, and 36 adenocarcinomas of urachal origin or located in the dome of the urinary bladder. A study of 5,324 bladder tumors in the registry of the American Urologic Association disclosed 69 adenocarcinomas.[2] Some adenocarcinomas of the urinary bladder produce mucin; those which produce large amounts of mucin are classified as mucinous adenocarcinomas, or as gelatinous or colloid carcinomas. Microscopically, one encounters in such neoplasms adenocarcinomatous structures together with distended acini which are filled with a homogeneous or slightly granular basophilic material. At times the lining cells are flattened by the pressure of the intracystic mucin, are degenerated, or are desquamated into the masses of mucin. Within the cystic spaces and between the glandular structures, there are also tumor cells which still carry within their cytoplasm the secreted mucin in such amounts that their nuclei are compressed and crescent-shaped, the cells thus appearing signet-ring-like. Wheeler and Hill found mucin-producing adenocarcinomas in two normally placed urinary bladders, although in an illustration of a mucin-producing adenocarcinoma arising in an exstrophic bladder many signet-ring cells are depicted, infiltrating the tissues between tumorous glands.

In a previous study,[3] a series of mucinous adenocarcinomas was reported which consisted principally of signet-ring tumor cells and only a few glandular structures, either without cystic spaces filled with mucin or with only a few of these present. All were diffusely infiltrating carcinomas and often produced widespread metastases. In many instances these tumors were either not recognized clinically or diagnosed so late in the course of the disease that radical surgery could not be performed. Because of their microscopic appearance and their very malignant course, and to distinguish them from uncomplicated mucinous carcinomas, they were grouped and classified as signet-ring cell carcinomas.

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† Research in this Department is supported in part by the Michael Reese Research Foundation.

Among 271 carcinomas of the urinary bladder removed surgically at Michael Reese Hospital between 1949 and 1953, I found 10 adenocarcinomas. Of these, 4 proved to be metastatic tumors, 2 of which were primary in the fundus of the uterus and 2 in the sigmoid colon. Of the remaining 6, one mucin-secreting adenocarcinoma with only a few signet-ring tumor cells arose at the dome of the bladder and was considered to be of urachal origin. In the other 5 cases the tumors arose in the anterolateral and posterior walls of the bladder adjacent to the trigone. Of these, 3 were non-mucin secreting adenocarcinomas and 2 were mucin-producing. However, signet-ring cells in a substantial number were found in only one of these mucin-producing tumors. This case, together with another in which the tumor consisted principally of signet-ring carcinoma cells and in which necropsy findings were available, formed the basis of this study.

The first patient was a Negro, 60 years old, who had been admitted because of difficulty in starting urination, urinary incontinence, nocturia, frequency, and hematuria which became progressively more severe during the last 3 months of life. An episode of acute urinary retention preceded hospitalization. On examination the prostate gland was enlarged and an intravenous pyelogram disclosed a greatly elongated prostatic urethra. The urine was cloudy and contained numerous red blood corpuscles. The clinical diagnosis was carcinoma of the prostate. A suprapubic drain was inserted and transurethral resection of the prostate was performed. The patient developed a rising temperature and became lethargic. The blood urea nitrogen was 99.5 mg. per cent and the creatinine, 4.9 mg. per cent. He died 2 weeks following the operation and about 4 months after the onset of his symptoms.

Only the more important necropsy findings will be given. The outstanding changes were found in the kidneys and in the urinary bladder. Careful examination of the stomach and the intestinal tract failed to show gross or microscopic changes. There was bilateral pyelonephritis with abscesses and marked pyonephrosis. The prostate was enlarged, adherent to the seminal vesicles, firm, and on section finely granular and pinkish gray with tiny yellow foci of necrosis. The urinary bladder was contracted, irregular in contour, very firm, and almost rigid. Only the dome seemed uninvolved. The mucosa was reddish gray, granular, and thickened, and a few superficial ulcers were recognized. Prominent mucosal folds were discernible in only a few portions. They were firm, markedly elevated, and on section glistening and gelatinous. The entire trigone was superficially ulcerated and brown as if charred, with prominent vertical streaks. There was no tumor in the sense of redundant tissue protruding into the lumen; however, the mucosa was densely adherent to the underlying loose connective tissue and could not be lifted with the forceps. On section, the wall of the bladder was thicker than normal, yellowish gray, and had the same peculiar glistening appearance seen in the mucosal folds.

The orifices of both ureters were severely encroached upon by the adjacent thickened mucosa, although they still admitted a fine probe. The peritoneal surface was thickened and granular. In the areas of thickened mucosal folds the wall of the urinary bladder measured 2 cm. in thickness. The seminal vesicles were densely adherent to the bladder wall and thickened.

Microscopically, practically the entire urinary bladder was diffusely infiltrated by tumor cells, most of which, comparable to the constituents of a phlegmonous inflammation, had invaded the loose tissue between the mucosa and muscularis and massively involved the mucosa. They had invaded the musculature by extension along vessels and connective tissue septa, and the peritoneal surfaces. By far the most common tumor cell was a signet-ring-like cell with a clear or faintly basophilic cytoplasm and a compressed, crescent-shaped nucleus. McManus' periodic acid-Schiff's reagent counterstained with iron hematoxylin for nuclei and saturated aqueous solution of picric acid for cytoplasm, and Cowdry's modification of Mayer's mucicarmine stain were employed to demonstrate mucin. These cells, singly or in small groups, as well as massively, invaded all layers of the urinary bladder. Only isolated small glandular structures lined by cuboidal cells, with or without mucin production, were noted. Small or minute, well circumscribed areas consisting only of mucin were rare. Also present were cells with very little cytoplasm and darkly stained, round, or slightly irregular nuclei, sometimes resembling monocytes. At times their cytoplasm was completely obscured. These cells were present either singly or in small groups, or had intermingled with the signet-ring tumor cells and had invaded the mucosa and the connective tissue septa of the muscularis. They were found also in the lumina of blood vessels. Often transitions between signet-ring tumor cells and these monocyte-like cells could be demonstrated. When only little mucin was present in the former, the nuclei became more round and irregular and were noted more often in the centers of the cells.

Within the mucosa, sometimes more or less as isolated structures but more often intermingled with the signet-ring carcinoma cells, were seen rare transitional epithelial cells exhibiting variations in size and staining quality, with a number of atypical mitotic figures. Occasionally, these cells also could be observed in the subjacent musculature. In a few fields where the mucosa was still intact, rare cell nests of Brunn could be made out. Some of these disclosed early cyst formation within their centers. Thus, while the tumor consisted mainly of infiltrating carcinomatous signet-ring cells, a few tumorous glandular structures and transitional epithelial cells were encountered also. The

prostate gland and the seminal vesicles were invaded by the carcinoma cells. Though most of these were signet-ring cells, transitional epithelial cells also were seen very occasionally in these two organs.

The second patient, a white male, 50 years old, was admitted because of intermittent incontinence over a period of 6 months and hematuria occurring 1 week previously. On cystoscopic examination there was a flat tumor located on the left lateral wall of the bladder which seemed also to infiltrate the left ureteral orifice. The tumor was resected and the adjacent regions were fulgurated. Three months later it recurred. A cystoscopic examination disclosed an infiltrating tumor involving practically the whole urinary bladder and occluding the orifices of the ureters. The bladder was fixed to the surrounding tissues. A bilateral cutaneous ureterostomy was performed and the patient made a relatively good recovery. He was readmitted 4 months later because of impending uremia and died shortly thereafter.

Specimens taken for biopsy disclosed a very few adenomatous structures with and without mucin. However, there were many signet-ring tumor cells either invading the tissues in groups or as isolated units. Often, too, there were smaller and larger cystic spaces filled with signet-ring tumor cells. A number of darkly stained hyperchromatic nuclei with very little cytoplasm, which was often not recognizable, were encountered—cells similar to those previously described. Also, obvious transitional epithelial cells were seen, but whether these were tumorous or constituted remnants of the cell nests of Brunn was difficult to determine.

Although signet-ring cell carcinomas of the urinary bladder apparently have not been reported, it must be understood that these are obviously mucin-secreting adenocarcinomas in which tumor cells, which are not within glandular confinements but either isolated or in small groups, invade the tissues, the individual tumor cells still retaining mucin production. Thus one is bound to encounter glandular structures also, although these may be difficult to find. It is noteworthy that the dome of the bladder was not involved in the first case and only later in the disease in the second case. It is therefore clear that the tumors did not arise from the urachus. This is important since there have been a number of urachal mucin-secreting adenocarcinomas reported in the urinary bladder, of which some—as is to be expected—also disclosed signet-ring tumor cells.[4]

As has been recently re-emphasized by Wheeler and Hill,[1] adenocarcinomas of the urinary bladder, with or without mucin secretion, arise most probably from the cell nests of Brunn. One often encounters, in instances of chronic cystitis, central cystic degeneration of these cell nests. The transitional epithelial cells adjacent to the cystic spaces are transformed into cuboidal cells, probably by a process of dedifferentiation or reversal to a more primitive type of cell. Thus, in cystitis

cystica one usually encounters (1) compact cell nests of Brunn; (2) such nests with cystic degeneration in their centers, in which the innermost lining cells are cuboidal and are surrounded by transitional epithelial cells; and (3) prominent cystic or glandular structures lined only by cuboidal epithelial cells. Thus it seems that in some instances adenocarcinomas may arise from these structures. In favor of this mode of origin is the frequent finding of both transitional cell and adenocarcinomatous features in the same tumor, the latter predominating. This has been found in previously recorded instances of adenocarcinomas[5] and has been reported also by Wheeler and Hill. It is not clear why in some instances the glandular structures thus formed go on to produce mucin. It is of interest also that in my 2 cases of signet-ring cell carcinoma, transitional cell structures also were found.

Morphologically, this tumor in the urinary bladder is identical with signet-ring cell carcinomas found in the stomach, large intestines, gallbladder, and breast.[3] The signet-ring cells first diffusely infiltrate the mucosa and submucosa of a hollow organ and later the entire wall, simulating a phlegmonous inflammation. The clinical recognition of these tumors is difficult because of their lateral spread in the absence of a protruding new growth. Also, the short clinical course of the disease after the diagnosis has been made has been repeatedly emphasized. Often, too, the correct clinical diagnosis cannot be made. The same attributes are found in these carcinomas of the bladder. One patient died 4 months after the first sign of the disease; curiously enough, the clinical diagnosis was carcinoma of the prostate gland.

When these tumors arise from organs other than the urinary bladder, they produce widespread metastases. However, there was no metastasis in the one case which was necropsied, although the seminal vesicles and the prostate gland were invaded by tumor cells. Unfortunately, a necropsy could not be performed in the second case. Perhaps the absence of metastases in the first patient may be explained by the relatively short course of the disease, the patient dying of uremia with acute pyelonephritis.

In a previously reported study,[3] it was noted that signet-ring cell carcinomas produced metastases to the ovaries and the intestinal tract more frequently than other types of carcinomas arising in similar organs. Both of the patients with signet-ring cell carcinomas of the urinary bladder were males. From my previous experience I feel that special attention should be given in future cases to the ovaries and the intestinal tract for possible metastases.

Summary

Signet-ring cell tumors, usually primary carcinomas of the gastrointestinal tract, may occur also as primary carcinomas of the urinary bladder. They are diffusely infiltrating lesions which occlude the ureteral orifices apparently early in their course, causing destructive pyelonephritis. Because of early invasion of the submucosal loose tissue and lateral spread they are diagnosed late in the disease. Microscopically, they are mucin-secreting adenocarcinomas with a vast predominance of epithelial cells resembling signet rings because of their mucous globules and which, isolated or in small groups, comparable to a phlegmonous inflammation, invade the entire wall of the bladder. Glandular structures may still be recognizable, but sometimes are difficult to find. Neighboring structures may be invaded but metastases are apparently rare, probably because of the short course of the disease which terminates in uremia.

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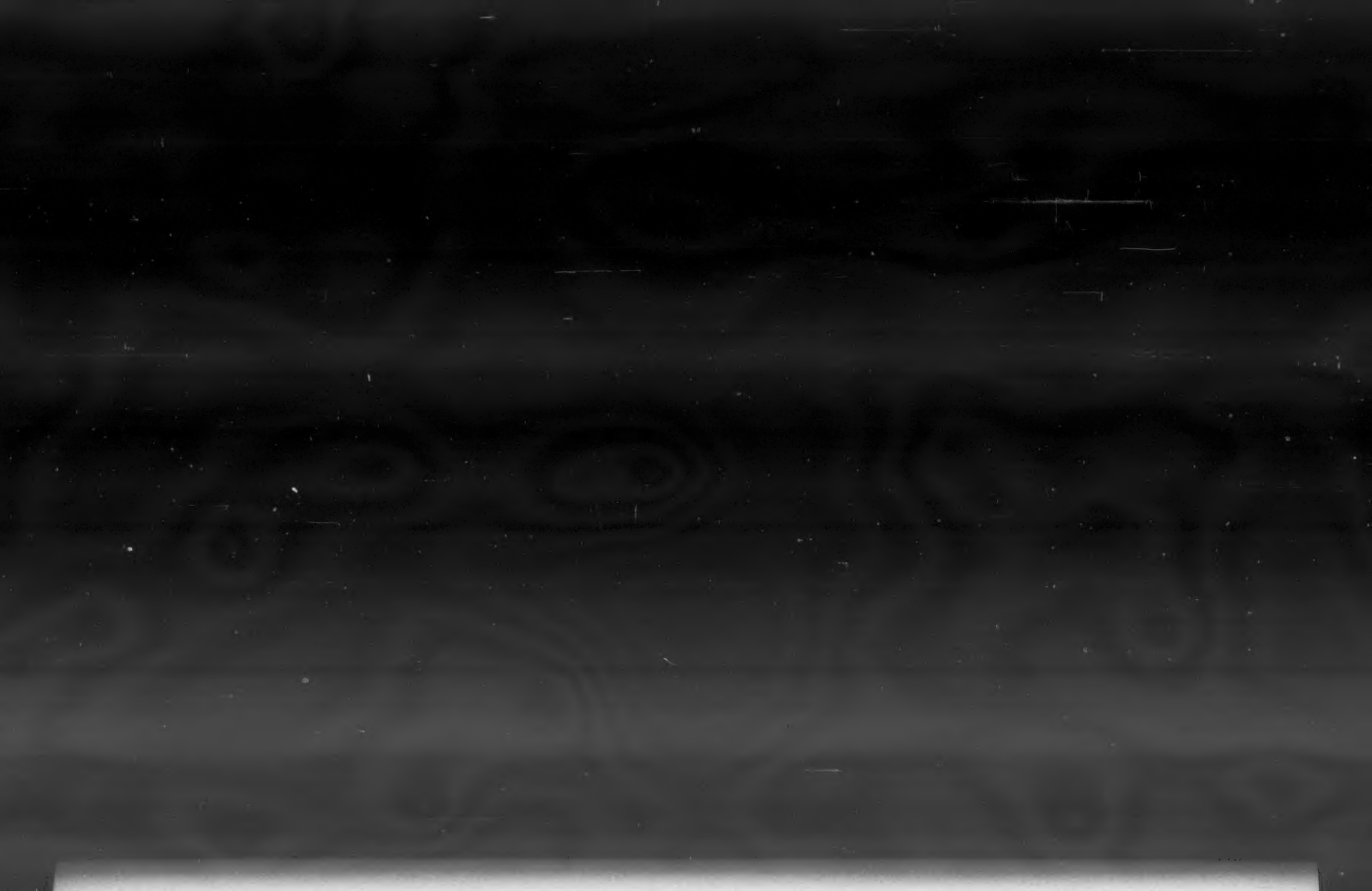
LEGENDS FOR FIGURES

FIG. 1. Glandular structures and signet-ring carcinoma cells. Hematoxylin and eosin stain. × 550.

FIG. 2. Signet-ring carcinoma cells. Iron hematoxylin stain. × 700.

FIG. 3. Signet-ring cells invading the mucosa of the urinary bladder. McManus' periodic acid-Schiff's reagent counterstained with iron hematoxylin. × 125.

FIG. 4. Signet-ring cells invading the wall of the urinary bladder. McManus' periodic acid-Schiff's reagent counterstained with iron hematoxylin for nuclei and saturated solution of picric acid for cytoplasm. × 150.



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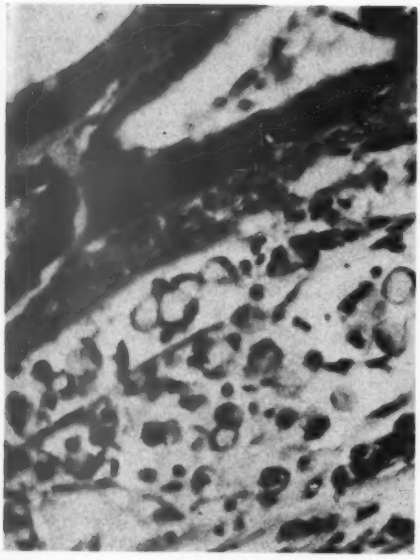

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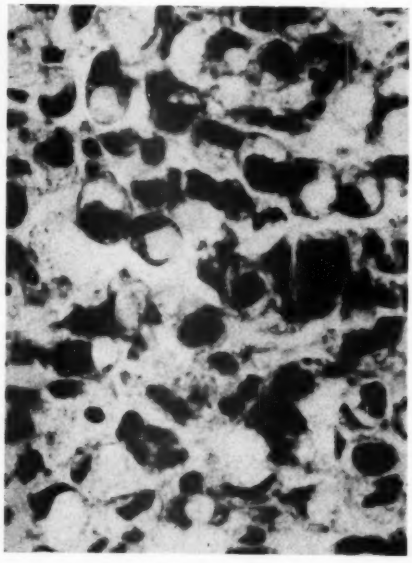

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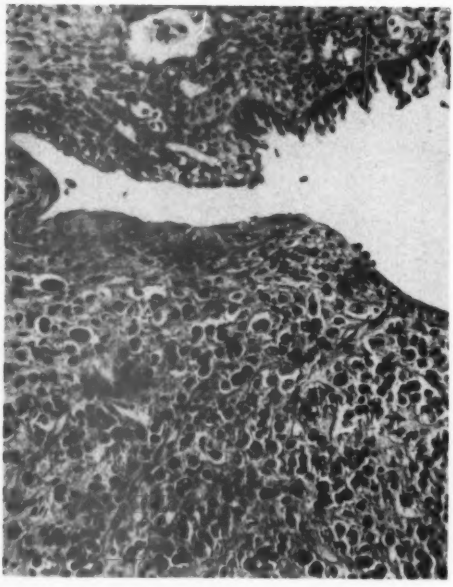

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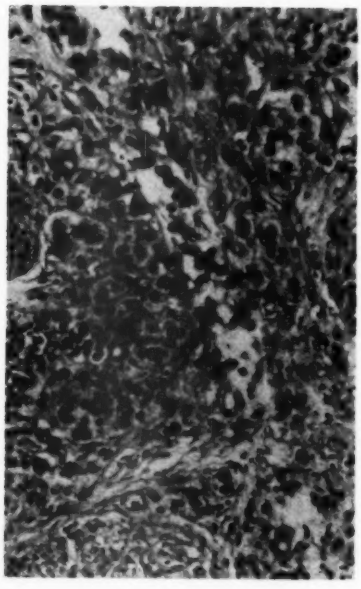

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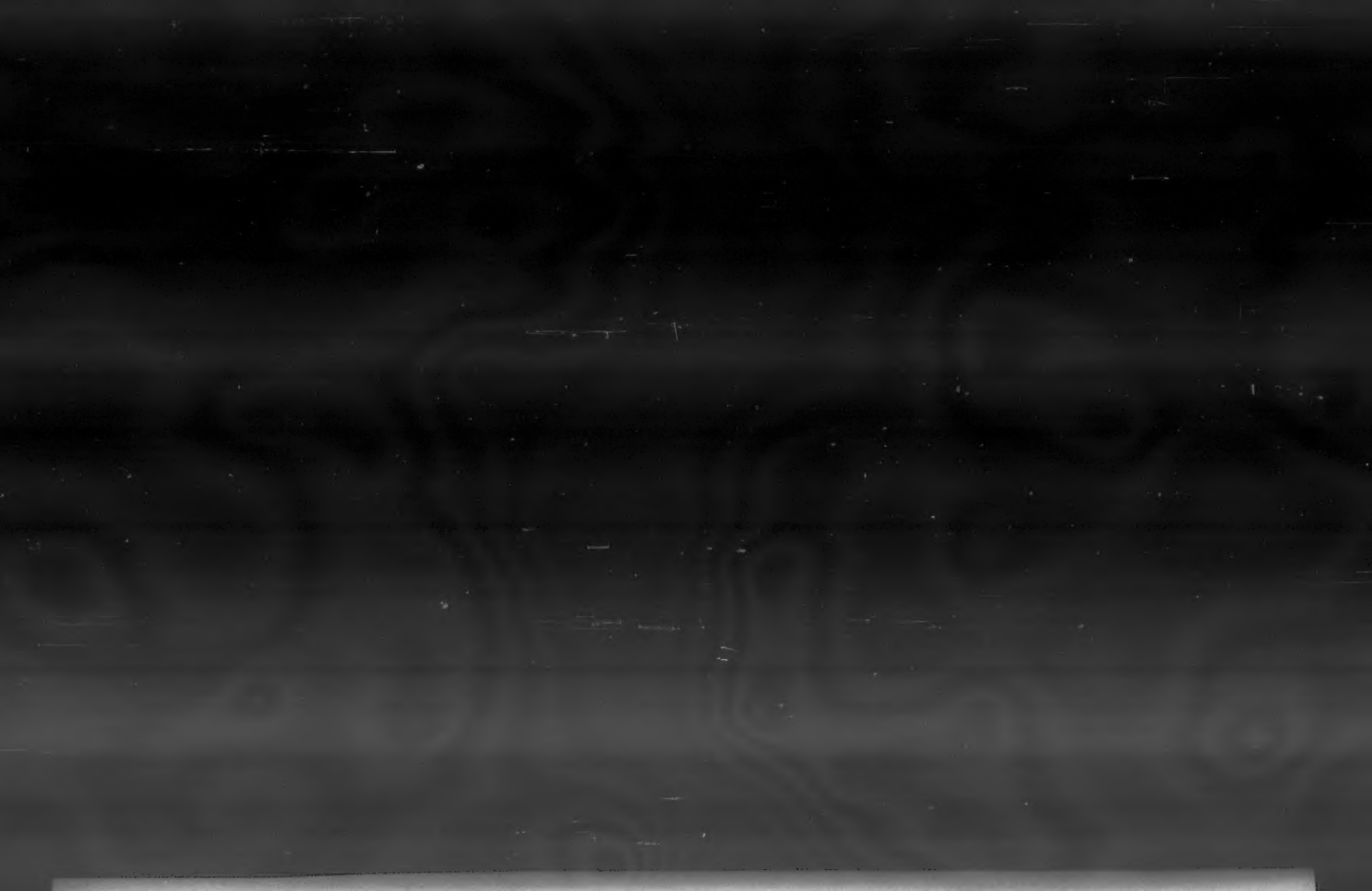
FIG. 5. Signet-ring cells invading the wall of the urinary bladder. McManus' periodic acid-Schiff's reagent counterstained with iron hematoxylin for nuclei and saturated solution of picric acid for cytoplasm. × 350.

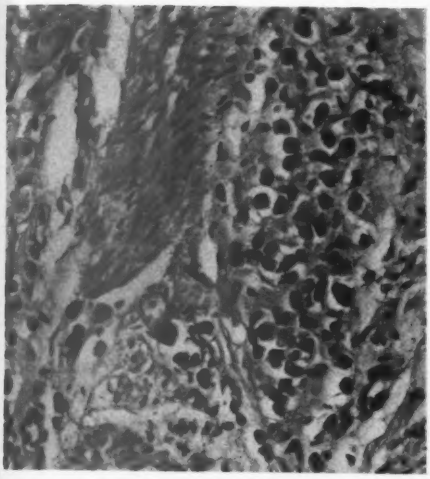
FIG. 6. Signet-ring carcinoma cells in vein. Hematoxylin and eosin stain. × 350.

FIG. 7. Involvement of lymphatics in nerve, and diffuse infiltration by large, round (carcinoma) cells. Iron hematoxylin stain. × 200.

FIG. 8. Large, round nuclei with very little cytoplasm invading the wall of the urinary bladder. McManus' periodic acid-Schiff's reagent counterstained with iron hematoxylin. × 400.

FIG. 9. Of note are the intermingling of signet-ring cells and the large, round nuclei of carcinoma cells. McManus' periodic acid-Schiff's reagent counterstained with iron hematoxylin. × 325.





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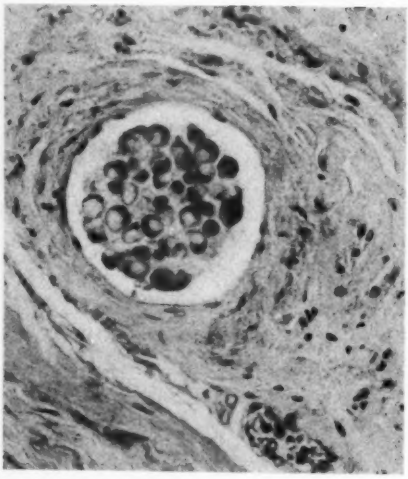

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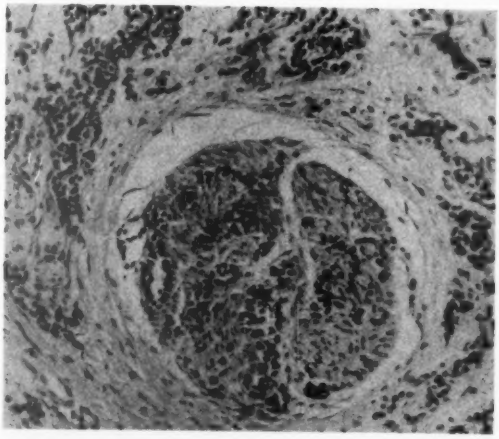

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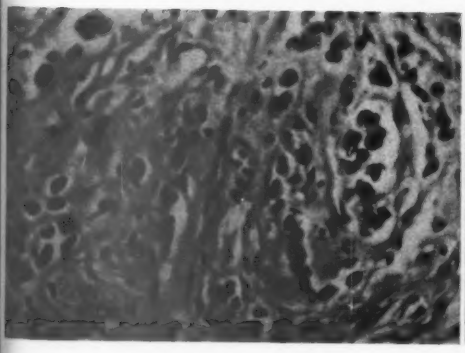

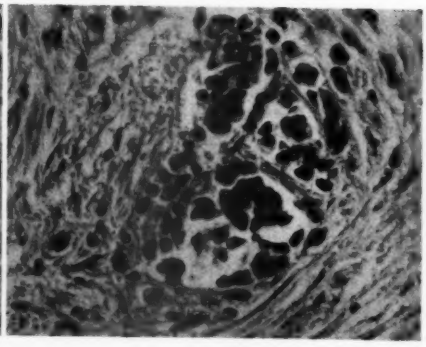

9

SELECTIVE RENAL VASOSPASM AND ISCHEMIC RENAL NECROSIS PRODUCED EXPERIMENTALLY WITH STAPHYLOCOCCAL TOXIN

Observations on the Pathogenesis of Bilateral Cortical Necrosis *

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Staphylococcal toxin, injected in large doses into the veins of rabbits, often brings about symmetric cortical necrosis of the kidneys,[1-4] and it also gives rise under suitable experimental conditions to ischemia of the renal cortex.[5,6] Yet its effects in these respects have not been extensively or systematically studied, and the findings thus far have failed to establish a relationship between the functional and anatomical changes or to provide a satisfactory basis for understanding the causation and pathogenesis of bilateral cortical necrosis.[7-10] In the present work rabbits given an arbitrarily chosen amount of staphylococcal toxin regularly manifested, after a brief interval, extreme congestion of the renal blood vessels together with complete functional ischemia. The congestion and ischemia proved transitory in approximately one half of the animals studied, the renal blood flow returning to normal within a few hours after the toxin had been given. In the remaining animals congestion and functional ischemia persisted, and changes developed in the kidneys which ranged from a more or less inconspicuous granularity and vacuolation of the cytoplasm of tubular epithelial cells, through necrobiotic changes of the epithelium of many scattered nephrons, to necrosis of all cortical structures and many of those in the medulla as well. The findings suggested that the character of the anatomical changes depended largely upon the extent and duration of the ischemia. Besides providing an example of the special reactivity of the renal vascular system to a circulating toxin, the findings have implications for the pathogenesis of bilateral cortical necrosis and perhaps also for that of other diseases characterized by acute renal ischemia and anuria.

Methods and Materials

In most of the experiments, young adult rabbits were first given a standard dose of staphylococcal toxin intravenously. Then, after vari-

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ous intervals, laparotomies were done in order to observe the kidneys directly, and a test was made of the flow of blood to the kidneys and other abdominal viscera by injecting India ink into the aorta and observing the blackening of the viscera resulting from its distribution. In addition, histologic studies were made of the kidneys of animals that had been sacrificed at various intervals following injection of the toxin, particular attention being paid to the distribution of India ink in the minute blood vessels and to the state of the tubular epithelium.

The staphylococcal toxin* had been made from a filtrate of *Staphylococcus aureus* (Wood 46 strain) grown in Leonard-Holme medium and was known to lyse rabbit erythrocytes and to bring about necrosis when injected into the skin of rabbits. To determine its hemolytic potency, a 1 per cent suspension of rabbit red cells was mixed with equal volumes of various dilutions of the toxin. The mixtures were incubated at 37° C. for 1 hour, the dilution of toxin that produced approximately 50 per cent hemolysis being recorded as the hemolytic titer. As tested in this way, the sample used had a titer of 1:320. The minimal lethal dose for mice was determined by diluting the toxin and injecting 0.2 cc. of the various dilutions intravenously into adult strain A mice weighing approximately 22 gm. Four mice were injected with each dilution, and the least dilution causing death within 5 minutes of at least 3 of 4 of the test animals was recorded as the minimal lethal dose. The sample used in these experiments had an M.L.D. of 1:10.

The standard dose of toxin used in the experiments on rabbits was 0.4 cc. of a 1:10 dilution in 0.9 per cent NaCl solution per Kg. of body weight. When the amount was increased to 0.8 cc. per Kg., the majority of the rabbits died within 30 minutes, whereas when the dose was reduced to 0.2 cc. per Kg., the ischemic effects, to be described, were not produced. Cats, on the other hand, were found to be far more resistant to the effects of the toxin, the lethal dose for a few adult animals averaging 2 cc. of undiluted toxin. In all cases the toxin was given as a single intravenous injection.

For control purposes, the toxin was specifically neutralized with antitoxin prepared in rabbits by Dr. Piersma and generously supplied by him. This was done by mixing equal volumes of toxin and antitoxin, and then incubating the mixture for 30 minutes at 37° C. The incubated mixture completely lacked hemolytic activity and was devoid of lethal effect in mice when injected intravenously in an amount ten times the M.L.D.

Young, market-bought, adult rabbits weighing 2 to 3 Kg. were used in most of the experiments. A few young adult cats and several albino rats also were used.

An India ink method, adapted from that employed by several other workers,[5,6] was used in determining the blood supply to the kidneys and other abdominal viscera. At various intervals after injection of the staphylococcal toxin, the animals were anesthetized with nembutal and ether. A laparotomy was then performed and the intestines deflected toward the right. The left kidney, with its artery and vein, could thus be observed directly in those animals without an excess of retroperitoneal fat. The aorta was then exposed about 0.5 cm. below the diaphragmatic hiatus, and 10 cc. of India ink, diluted 1:2 in saline solution, was slowly injected. The flow of India ink through the various abdominal viscera was observed directly. After 5 minutes, the animal was killed with ether; further scrutiny of the distribution of ink in the various viscera was then made, and blocks of the organs and tissues were taken for microscopic study.

* Supplied by Dr. H. D. Piersma of Lederle Laboratories, Pearl River, N.Y.

Results

Visible Renal Changes Following the Intravenous Injection of Staphylococcal Toxin into Rabbits and Other Experimental Animals

Initially, a laparotomized rabbit was given a single injection of staphylococcal toxin into a marginal ear vein with the aim of observing directly any alterations that the toxin might bring about in its kidneys. It was known from the studies of others that under suitable experimental conditions the toxin is capable of producing bilateral cortical necrosis and also alterations in the renal blood flow.[1-6] Under direct observation of the anesthetized animal, it was seen that during the first 15 to 20 minutes following injection of the toxin the exposed kidney remained unchanged in size and color. Soon afterwards, however, the organ abruptly began to swell and its normal ruddy hue to deepen, so that within a few minutes the kidney had become about one third larger than normal and was intensely cyanotic. By this time, the renal vein, which in the rabbit is normally a large vessel, was seen to be in extreme spasm throughout its length, and the pulsation of the renal artery could no longer be made out. The spasm of the vein persisted, as did the swelling and cyanosis of the entire organ, these manifestations still being marked 60 minutes after injection of the staphylococcal toxin. Careful inspection of the other abdominal viscera, together with the blood vessels serving them, failed to reveal any abnormality during this period.

To learn more about the phenomenon just described, India ink was injected into the upper abdominal aorta of a rabbit that had been given 0.4 cc. of a 1:10 dilution of staphylococcal toxin 2 hours previously. As soon as the injection of ink was started, the liver, gastrointestinal tract, and posterior abdominal wall were seen to blacken, and by the time the injection had been completed these organs and tissues were coal black; the kidneys, however, failed to blacken (Fig. 1). Sections of the liver and spleen, made following sacrifice of the animal, showed uniform blackening, but, apart from rare punctate flecks, no ink was seen in multiple sections of both kidneys.

For control purposes, a normal rabbit of comparable size was next given 0.6 cc. per Kg. of 0.9 per cent NaCl solution intravenously, with laparotomy and injection of the India ink 60 minutes later. As the India ink was injected, the kidneys and other abdominal viscera blackened concurrently, and soon all were jet black (Fig. 2). There was uniform blackening of the cortex and medulla of both kidneys, as sagittal sections disclosed.

The observations just described were confirmed repeatedly in subsequent experiments. For example, in one experiment 8 rabbits were each given 0.4 cc. of a 1:10 dilution of staphylococcal toxin per Kg., with inspection of the renal vessels 40 to 120 minutes later. In every instance the renal vein was seen to be in spasm throughout its length, while the renal arteries did not pulsate. At the end of the 2-hour period India ink was injected into the abdominal aorta of each animal. Again, without exception the kidneys either failed to blacken or displayed only a few black flecks on section owing to the filling of occasional intralobular arteries with ink, while the other abdominal viscera regularly blackened intensely, precisely as had those of the rabbits used for Figures 1 and 2. In 10 control rabbits given 0.6 cc. per Kg. of 0.9 per cent NaCl solution into an ear vein, with injection of India ink into the aorta 2 hours later, the renal vein remained filled with blood in each animal, while the renal artery continued to pulsate, and the kidneys all blackened intensely and as promptly as did the other abdominal viscera when India ink was injected into the abdominal aorta. Figure 3 shows the kidneys from 2 of the control rabbits and 2 of the toxin-injected rabbits following injection of the India ink, the findings being representative of those in each group.

In several further experiments, 10 cc. of diluted India ink was injected into a marginal ear vein of each of 4 control rabbits and into that of 4 rabbits that had received the standard dose of staphylococcal toxin 2 hours previously, the variation in method being employed to avoid anesthesia and laparotomy. The kidneys of all control rabbits, examined following sacrifice 5 minutes after injection of the ink, were regularly grayish black, as were the other abdominal viscera. The kidneys of the toxin-injected rabbits, by contrast, regularly remained swollen, cyanotic, and devoid of ink, though the rest of the abdominal viscera were blackened in each instance.

Additional experiments were made to learn whether similar changes would follow injection of staphylococcal toxin into animals of other species. In each of 2 young adult cats, 20 cc. of India ink was injected into the aorta approximately 2 hours after 0.5 cc. of the undiluted toxin had been injected intravenously into the left femoral vein. The results were fully comparable to those obtained in rabbits, the kidneys regularly remaining reddish blue and swollen, while the rest of the abdominal viscera were blackened. The findings were essentially the same in 6 young adult white rats given 0.1 cc. of toxin per Kg. into a tail vein.

Responsibility of the Toxin for the Renal Ischemia

Several experiments were made next with the aim of learning whether the toxin itself could be held responsible for the observed effects. The findings are summarized in Table I. From this it can be

TABLE I

Effects of Staphylococcal Toxin and Control Materials on Renal Blood Flow of Rabbits, as Determined by the India Ink Method *

Materials injected intravenously	Amount injected	No. of rabbits	Outcome of India ink test made 60 minutes after injection of test material†
			Blackening of kidneys
Toxin-antitoxin mixture‡	0.8 cc./kg.	4	Complete in all animals
Toxin, at 60° C. for 1 hr.§	0.4 cc., 1:10 dil./kg.	4	Complete in all animals
Toxin, at 80° C. for 1 hr.‡	0.4 cc., 1:10 dil./kg.	4	Complete in all animals
Leonard-Holme culture medium	0.6 cc./kg.	4	Complete in all animals
0.9% NaCl solution	0.6 cc./kg.	10	Complete in all animals
Staphylococcal toxin	0.4 cc., 1:10 dil./kg.	8	Nil in all animals

* For details of the India ink method, see the text.

† The abdominal viscera other than the kidneys were regularly blackened in all the animals.

‡ Equal amounts of undiluted toxin and antitoxin (rabbit serum) were mixed and incubated at 37° C. for 30 minutes. The mixture proved non-hemolytic for rabbit cells, and was innocuous when injected intravenously in mice in amounts of 0.2 cc.

§ The preparation was neither hemolytic for rabbit cells nor lethal for mice when injected intravenously in amounts of 0.2 cc.

seen that a mixture containing ten times the standard dose of toxin and sufficient antitoxin to render it non-hemolytic and non-lethal for mice had no influence on the renal blood flow of rabbits, as determined by the India ink test given 1 hour after injection of the neutralized mixture. The same proved true of filtrate preparations that had been rendered "non-toxic" by heating at 60° C. and 80° C., respectively, for 1 hour, of culture medium of the type in which the toxin had been produced, and of 0.9 per cent NaCl solution. The unheated filtrate, however, induced renal ischemia in all 8 rabbits given the standard amount, the kidneys in each instance failing to blacken following injection of the India ink while the other abdominal viscera regularly did so. Although by no means conclusive, the findings are seen to be consistent with the possibility that the toxin itself is responsible for the ischemia.

Extent and Duration of the Renal Ischemia

The results of studies to determine the extent and duration of the renal ischemia that follows injection of staphylococcal toxin into rabbits are summarized in Table II. From this it can be seen that the 5

TABLE II

Renal Blood Flow at Various Intervals Following Injection of Staphylococcal Toxin in Rabbits

Intervals between injection of toxin* and application of India ink test	No. of rabbits	Renal blood flow in the various test animals as determined by the India ink test†					
a. 5–15 min.	5	++++	++++	++++	++++	++++	
b. 20–40 min.	5	++	++	+	0	0	
c. 40–120 min.	15	0	0	0	0	0	0
		0	0	0	0	0	0
		0	0	+			
d. 3–6 hours	10	0	0	+	+	++	++
		++++	++++	++++	++++		
e. 2 days	2	++++	++++				
f. 4 days	2	++++	++++				
g. 7 days	1	++++					

* 0.4 cc. of 1:10 dilution of toxin/kg. injected into marginal ear vein of each animal.

† Blackening of the liver, stomach, intestines, spleen, posterior abdominal wall, and the tissues of the lower limbs was regularly observed in all animals.

The results of the India ink tests were graded and recorded for each animal as follows:

++++ = normal renal blood flow. In these instances the kidneys prior to injection of the India ink were normal in size and bright pink; they blackened almost coincidently with the start of the India ink injection, and ink was regularly seen in copious amounts admixed with blood in the veins. On section, the cortices and medullae were uniformly gray-black. Microscopically, ink was seen within virtually every glomerulus and was distributed uniformly throughout the peritubular capillaries and other vessels. The findings were precisely the same as in control rabbits that had not been given toxin.

++ = moderately diminished renal blood flow. The kidneys were swollen and cyanotic prior to injection of the India ink; afterwards they became mottled gray-black. The renal vein was collapsed or partially spastic, but small amounts of India ink admixed with blood could be seen entering it from the renal side. Numerous flecks of black were present on the cortical surfaces owing to the presence of ink in the intralobular arteries. Microscopically, ink admixed with blood was found in the glomeruli and peritubular capillaries in numerous thin, radially arranged segments of the cortices, and in approximately half the juxtamedullary glomeruli and their attendant vasa recta. The bulk of the cortical glomeruli and the remaining juxtamedullary glomeruli, together with the peritubular capillaries draining them, were greatly engorged with blood which was devoid of admixed ink.

+ = markedly diminished renal blood flow. The findings were essentially like those in the animals graded ++ except that the ink perfused only rare radial segments of the cortex and only about one fifth the juxtamedullary glomeruli and their attendant vasa recta.

0 = virtually complete cessation of renal blood flow. Apart from the rare finding microscopically of a few particles of ink within the arcuate arteries, there was no evidence of renal circulation.

animals that were tested with India ink during the first 15 minutes following injection of the toxin had intensely blackened kidneys, the

findings indicating normal renal blood flow. However, the 5 animals given the India ink test during the period of 40 to 120 minutes following injection of the toxin displayed marked or virtually complete renal ischemia; and this was true as well in the 15 animals tested during the period of 40 to 120 minutes, and in 6 of the 10 animals examined during the interval of 3 to 6 hours following injection of the toxin. By contrast, the renal blood flow, as determined by the India ink test, was normal in 4 of the 10 animals tested 3 to 6 hours after the toxin had been given, and so, too, in the 5 rabbits examined on the second, fourth, and seventh days. In all animals the abdominal viscera other than the kidneys, and the tissues of the lower limbs as well, regularly blackened uniformly and intensely immediately following the injection of India ink into the aorta.

All rabbits in the experiments outlined in Table II remained lively and apparently normal throughout the periods of observation. In retrospect, it seems particularly striking that the 6 animals with greatly diminished renal blood flow in the group sacrificed during the period of 3 to 6 hours following injection of the toxin were quite as lively prior to laparotomy as were the 4 animals with normal renal blood flow. It was noteworthy also that the rabbits sacrificed, respectively, at 2, 4, and 7 days following injection of the toxin had likewise remained lively and, apart from a transitory oliguria, were devoid of signs of illness throughout the periods of observation. But this was not uniformly so, as the observations of the next section show. More than half the rabbits that had been given the standard amount of staphylococcal toxin and then were allowed to survive for 6 hours or longer, sickened abruptly and died during the period of 9 to 36 hours following injection, the anatomical changes suggesting that the renal ischemia in these fatal instances must have been prolonged.

The findings thus far given have made it plain that the renal ischemia was selective. It did not become manifest during the period of 5 to 15 minutes following injection of the toxin but developed regularly 20 to 40 minutes after injection. It usually persisted for more than 1 hour, and often proved transitory. The renal blood flow was normal, as judged by the India ink test, in a considerable proportion of the animals tested 3 to 6 hours following injection of the toxin and thereafter. It may be noted here that the transitory character of the renal ischemia which may follow the intravenous injection of staphylococcal toxin in rabbits was not apprehended in previous studies.[1-6] Furthermore, the observations just mentioned, while agreeing with

those of Trueta *et al.*[5,6] on the selective nature of the effect of staphylococcal toxin on the renal blood vessels, differ in several other particulars. While direct comparison of the findings is rendered difficult by the fact that the observations of Trueta *et al.* have not been recorded systematically, it is obvious that at least two striking discrepancies exist. First, Trueta *et al.* recorded a marked blanching of the kidney within a few minutes after intravenous injection of staphylococcal toxin, whereas in the present work the kidneys regularly became swollen and deeply cyanotic after 20 to 40 minutes. Secondly, Trueta *et al.* described a pulsatile stream of bright red blood in the renal vein shortly after injection of the toxin, an observation that was not made in any of the animals of the present work. The anatomical findings, to be given further on, provide additional evidence on these and other controversial points.

STRUCTURAL CHANGES IN THE KIDNEYS IN RELATION TO ALTERED RENAL BLOOD FLOW IN RABBITS GIVEN STAPHYLOCOCCAL TOXIN

Several previous workers have shown that structural changes often develop in the kidneys of rabbits and other animals given staphylococcal toxin intravenously.[1-4] With the aims of studying such changes under the standardized conditions of the present investigation and of correlating these with the alterations in renal blood flow, gross and microscopic examinations were made of the kidneys of 44 rabbits that had died or were sacrificed at various intervals after the toxin had been administered. In 36 of the animals (those of Table II) the renal blood flow had been determined by means of the India ink method immediately prior to sacrifice. In every instance the findings were essentially the same in both kidneys of each rabbit. A detailed description of the gross and microscopic findings, together with interpretations of the changes, will now be given.

Normal Renal Blood Flow in Rabbits Examined 5 to 15 Minutes Following Injection of Staphylococcal Toxin

No alterations were noted in the kidneys of the 5 rabbits killed within 5 to 15 minutes after toxin had been injected into an ear vein (and within 5 minutes following injection of India ink into the aorta); in each instance (group c, Table II) the glomerular and peritubular capillaries were all more or less uniformly perfused with India ink, as was the case when uninjected control rabbits, or those given saline solution intravenously instead of toxin, were examined similarly. The microscopic findings provided additional evidence that the renal blood

flow remained normal during the period of 5 to 15 minutes following injection of the toxin.

"Stagnation Hyperemia" and Partial or Complete Ischemia in Rabbits Examined 20 to 120 Minutes Following Injection of Staphylococcal Toxin

Five rabbits were examined during the period of 20 to 40 minutes following injection of the toxin. The renal blood flow, as judged by the India ink method, was graded 2 plus in 2, 1 plus in one, and zero in 2 (explanation given in footnote to Table II). The kidneys of all animals of this group, examined at laparotomy prior to injection of the India ink, were swollen and cyanotic. In the 2 animals in which the renal blood flow was graded 2 plus the kidneys became mottled and gray-black following injection of India ink into the aorta. Histologically, India ink was seen in nearly all of the arcuate arteries and in most of the juxtamedullary glomeruli; furthermore, it filled about half the intralobular arteries, the afferent arterioles, the capillaries of the glomeruli supplied by them, and the peritubular capillaries and venules draining these structures as well. The rest of the blood vessels were greatly distended with blood, and little or no ink was admixed with it. Hence the cortices were curiously mottled, with ink-filled radially arranged segments alternating more or less at random with other radial segments in which the vessels were distended with blood but devoid of ink. The findings were similar in the one animal in which renal blood flow was graded 1 plus except that only about one fifth of the juxtamedullary glomeruli and only a few radial segments of the cortex contained ink, while all remaining vascular channels were distended with blood. The point deserves emphasis that although the amount of India ink present in the juxtamedullary glomeruli and vasa recta in the 3 animals, graded 2 plus and 1 plus, was distinctly greater than that perfusing the cortical glomeruli of the same organs, still the perfusion was a great deal less than that seen in the juxtamedullary glomeruli of control animals.

The possible relationships of the hyperemia and partial ischemia noted in the instances just described will be considered further on, after a description of the findings in a number of rabbits in which ischemia was virtually complete.

In the 2 animals in which the renal blood flow was graded zero the kidneys did not darken following injection of the India ink. Histologic studies disclosed that the majority of the glomeruli were greatly distended with blood, as were the peritubular capillaries and the intra-

lobular veins; India ink virtually was absent from the distended blood vessels, though here and there small flecks of it, admixed with blood, were found. The renal parenchyma was unchanged.

The findings in the 15 rabbits sacrificed in the interval from 40 to 120 minutes following injection of the toxin (group c, Table II) were quite like those in the rabbits graded zero in group b already described. Renal blood flow, as determined by the India ink method, was virtually nil and an extreme hyperemia of the majority of the small vessels characterized the histologic picture. Again India ink was absent from nearly all distended blood vessels, though it was present in very rare juxtamedullary glomeruli and their subjacent vasa recta. A colloidal precipitate, staining pink in hematoxylin and eosin preparations, was seen in Bowman's space surrounding some of the glomerular tufts and in the lumina of some of the proximal convoluted tubules. As in the animals of group b already described, interstitial edema was minimal.

The fact that India ink failed to enter the bulk of the small blood vessels in the kidneys of the animals of group c, while at the same time these vessels were greatly distended with blood, seemed to indicate plainly that the columns of blood were stagnant. The findings in 3 of the animals of group b provided further evidence on the point. In these the ischemia was only partial, and many of the juxtamedullary glomeruli and their attendant peritubular capillaries and vasa recta contained ink admixed with small amounts of blood, as did nearly all the blood vessels in a number of thin, radially arranged segments of cortical tissue. Yet in these organs also, blood distended the many small vessels that were devoid of ink. The findings as a whole made it obvious that columns of stagnant blood occupied the vessels in some segments of the kidneys while the vascular channels in other segments remained open. Similar alterations in renal blood flow have been noted in animals given vasopressin,[11] and in others following electrical stimulation of the cerebral cortex,[12] a non-uniform vasoconstriction primarily of venules or veins being held responsible for the effects in each instance.

"Stagnation Hyperemia," Functional Ischemia, and Ischemic Tubular Changes in Animals Sacrificed 4½ and 6 Hours Following Injection of Staphylococcal Toxin

Of the 10 rabbits sacrificed 3 to 6 hours following injection of the toxin, 2 displayed almost complete cessation of renal blood flow, 4 had varying degrees of severely restricted blood flow, and 4 others had virtually normal flow, as judged by the India ink method. The

structural changes found in the kidneys of the 2 animals in which renal blood flow had virtually ceased differed markedly from those found in the other animals. Hence the findings in these 2 animals will be considered apart from those encountered in the 8 remaining rabbits killed during this period.

When first viewed at laparotomy the kidneys of these 2 animals were turgid and cyanotic, and their gross appearance, as seen through the capsules, did not change following injection of India ink into the aorta. The distribution of blood and the perfusion of India ink, as observed in the histologic preparations, were almost identical in the 2 animals. In the arcuate and intralobular arteries and in the afferent arterioles a small amount of blood, approximating that found in corresponding vessels of normal control rabbits, was seen. The cortical and juxtamedullary glomeruli, however, were greatly engorged with blood and the peritubular capillaries were similarly distended. In preparations 7 μ in thickness, virtually no India ink could be seen in the sections, apart from rare flecks in arcuate arteries. In sections 20 μ thick, however, about 1 in 10 of the juxtamedullary glomeruli and their attendant vasa recta contained small amounts of ink.

In the animal sacrificed at 4½ hours, the glomeruli appeared normal apart from the engorgement previously described. The cytoplasm of the proximal tubular epithelial cells, however, was swollen and vacuolated, and numerous hyaline droplets replaced the mitochondria. More extreme epithelial changes were seen in the medullary portions of the loops of Henle, where the cells were greatly vacuolated and the lumina of the tubules were distended with pink colloid precipitate. In the animal sacrificed at 6 hours, the glomerular basement membranes were thickened and hazy, and the nuclei of the cortical glomerular tufts were all intensely pyknotic. Furthermore, the cells of the convoluted tubules showed early coagulation necrosis, their nuclei being pyknotic and occasionally karyorrhexic, while their cytoplasm was acidophilic and granular. The larger blood vessels in both animals appeared normal histologically; none contained thrombotic material and the same was true of the glomerular and peritubular capillaries.

The findings in these 2 animals were interpreted as showing persistence of the virtually complete stagnation hyperemia and functional ischemia which, as the previously studied instances have shown, must have begun within 20 to 40 minutes following injection of the toxin, and remained complete during the period from 40 to 120 minutes. In addition, there were structural changes in the kidneys—notably a granularity and vacuolation of tubular epithelium in one instance, necrobiotic changes in the tubules and glomeruli with alterations in the basement membranes in the other—which were interpreted as being consequent to the persisting ischemia. Similar but much more extreme anatomical changes were encountered in animals to be described.

Partial or Complete Return of Renal Blood Flow in Rabbits Examined 3 to 6 Hours after Injection of the Toxin

In 4 animals, renal blood flow as judged by the India ink test was markedly restricted in one rabbit (graded 1 plus) and moderately

restricted in 3 others (graded 2 plus). Prior to the injections of India ink, the kidneys of these animals were swollen and mottled pink and blue; afterwards, although the external cortex appeared largely unchanged, it became plain on sectioning the organs that ink had entered and blackened the juxtamedullary areas to some extent, particularly in the 3 animals graded 2 plus. In general, the cortical glomeruli and peritubular capillaries were found in histologic preparations to be engorged with blood but devoid of ink, though occasional radially arranged cortical segments, comprised of areas supplied by individual intralobular arteries, were perfused with ink admixed with blood; these cortical segments were fairly numerous in the 3 rabbits graded 2 plus though rare in the animal graded 1 plus. The glomeruli appeared normal apart from the hyperemia already mentioned. The cytoplasm of cells of the proximal convolutions generally showed coarse granularity, loss of the rod-like arrangement of the mitochondria, and occasional large, clear, cytoplasmic vacuoles, though the nuclei retained their normal vesicular appearance. A granular precipitate often was present in the lumina of the loops of Henle and of the distal convoluted tubules, and occasionally in the collecting tubules.

All 4 remaining animals showed an entirely normal blood flow as judged by the results of the India ink test. Prior to injection of the ink, the kidneys were ruddy and only slightly swollen, if at all; afterwards, they were intensely blackened. Microscopically, ink was distributed more or less uniformly throughout the blood vessels of the kidneys, more of it within the cortical and juxtamedullary glomeruli and in their attendant capillaries than within the larger vessels, the results as a whole being quite comparable to those following the perfusion of the kidneys of normal animals with India ink under similar conditions. Again there was moderate swelling, granularity, and vacuolation of the cells of proximal convolutions, with loss of the normal rod-like arrangement of the mitochondria; precipitated granular material was found mainly in the lumina of the loops of Henle and in those of the distal convoluted tubules.

Since virtually complete renal ischemia had been found in all 15 rabbits given the standard dose of toxin and examined 40 to 120 minutes later (Table II), the findings in the 8 animals just considered were interpreted as indicating respectively a partial and a complete return of blood flow to kidneys that had previously been totally ischemic. The granularity and vacuolation of tubular epithelium were considered to be relatively slight and probably reversible cellular changes resulting from the ischemia. It is of interest in this relation that the return of blood flow, while it had become complete in 4 rabbits of this

group, was only slight to moderate in the rabbits graded 1 and 2 plus. Furthermore, it was manifest that the return of blood flow to the various segments of the kidney was not uniform, for only a small proportion of the juxtamedullary glomeruli and their attendant vasa recta and only rare intralobular arteries and their dependent vessels were filled with ink in the 2 rabbits of group d that were graded 1 plus, approximately half the juxtamedullary glomeruli and their vasa recta and the segments of cortex supplied by numerous intralobular arteries were perfused in the 2 rabbits graded 2 plus, and all the glomeruli and their attendant capillaries were uniformly perfused in the kidneys of the 4 rabbits graded 4 plus. The findings in the animals in which the return of renal blood flow was incomplete were quite similar to those encountered at the onset of the ischemia (for example, the results in group b, Table II) and it seems logical to suppose that the factors responsible for the irregularity of renal blood flow at the onset of the ischemia were likewise operative as the abnormal process subsided. More will be said about these factors in the discussion.

Bilateral Renal Necrosis in Rabbits Dying 9 to 38 Hours Following Injection of the Toxin

In all 9 rabbits dying during the period 9 to 38 hours after injection of the toxin, extensive bilateral renal necrosis was found, precisely like that encountered in the naturally occurring disease of human beings.[7-10]

In the one animal dying 9 hours after injection of the toxin virtually the entire renal cortex and much of the medulla were seen to be dull gray and opaque, and were recognized as being necrotic. In the necrotic areas, the cells of the glomeruli and of the adjacent convoluted tubules, blood vessel walls, and interstitial tissues displayed acidophilic coagulation of cytoplasm and pyknosis of nuclei upon microscopic examination, while the basement membranes of the glomerular loops and tubules appeared generally swollen, hazy, and eosinophilic. The cells in approximately 1 in 10 of the juxtamedullary glomeruli remained essentially unchanged, and those of the convoluted tubules in the immediate vicinity of the viable juxtamedullary glomeruli did not manifest the coagulation necrosis seen elsewhere; but they often displayed granularity and vacuolation of the cytoplasm without alterations in the nuclei. Occasional hemorrhages were seen in both cortex and medulla. The arcuate arteries appeared normal and usually were empty. In this animal intralobular arteries also were normal at their sites of origin, and so, too, were the short segments of these arteries proximal to the boundary between the viable and ne-

crotic renal parenchyma. Here, however, the vessel walls abruptly became swollen and often hemorrhagic, while distally—that is to say, within the necrotic zones—the vessel walls were coagulated and obviously necrotic. Leukocytes were seen emerging from the capillary tufts in the surviving juxtamedullary glomeruli and also from the walls of the intralobular arteries in the swollen segments of those vessels that traversed the boundary between the viable and necrotic renal tissue.

In the 8 animals dying during the period 12 to 38 hours following injection of the toxin, the anatomical changes were much like those just described. In every instance the necrosis involved virtually the entire cortex, including glomeruli, tubules, blood vessels, basement membranes, and interstitial tissues, and usually the bulk of the medulla as well, though the epithelium of the renal pelvis, the capillaries of occasional juxtamedullary glomeruli, together with the convoluted tubules associated with them and some of the collecting tubules and supporting structures comprising the pyramids, were regularly spared. Indeed in one rabbit approximately 50 per cent and in 5 others 10 to 20 per cent of the juxtamedullary glomeruli were engorged with blood, their cells with those of the tubules and adjacent supporting structures remaining largely unchanged. In the 2 remaining animals of this group, however, virtually all the glomeruli, juxtamedullary as well as cortical, were necrotic, while the necrosis in the more extreme case involved practically the entirety of each kidney except for the epithelium of the renal pelvis and small segments of the pyramids. A more detailed description of the findings in these 8 animals follows.

Rabbit 12–44. Rabbit 12–44 died approximately 9 hours after injection of the toxin. The kidneys were swollen and cyanotic, and large amounts of dark blood flowed from their cut surfaces. There was marked congestion of both cortex and medulla, the usual contrast between the two regions being virtually absent. Microscopically, the small blood vessels were greatly engorged, and the cells of virtually all tubules and glomeruli—those in the juxtamedullary areas as well as those in the outer cortex—were necrotic, the cytoplasm of the epithelial cells being coagulated and eosinophilic and the nuclei being either pyknotic, karyolytic, or karyorrhexic. Likewise the basement membranes and the walls of the blood vessels throughout the entire cortices were swollen, hazy, and acidophilic; they were obviously necrotic. Necrosis was likewise widespread in the pyramids. Here the loops of Henle, collecting tubules, and adjacent vascular and supporting structures were involved almost uniformly. But scattered through the pyramids were small foci in which the epithelial cells of the tubules and the supporting structures were unaltered, the border between the living and non-living tissue being quite sharp. These small foci of viable medullary tissue were often adjacent to one of the relatively few juxtamedullary glomeruli in which the cells of the tufts remained unchanged. Such glomeruli were invariably engorged as were their subjacent vasa recta, and small hemorrhages were sometimes seen around them. Leukocytes were seen emerging from many of

these vessels, but on the whole the inflammatory reaction was slight and limited to foci in the interstitial tissues of the medullary and juxtamedullary areas. The walls of the intralobular arterioles were uniformly necrotic and this was frequently true also of the arcuate arteries. None of the vessels contained thrombi.

Rabbit 12–45. Rabbit 12–45 died approximately 12 hours after injection of the toxin. The gross and microscopic findings were almost precisely like those in rabbit 12–44; congestion was perhaps more extreme and there were numerous hemorrhages in the cortex.

Rabbit 13. Rabbit 13 died approximately 18 hours after injection of the toxin. The kidneys were alike and slightly enlarged. The capsules came away readily from the cortices which externally were smooth, opaque, and mottled brown and gray. On cut section the mottled cortices were seen to be separated from the congested medullae by thin, white, serpiginous lines which ran somewhat irregularly, but generally parallel with the cortex. Microscopically, virtually the entire cortex of each kidney was necrotic. The tubules were recognizable as eosinophilic, structureless masses with peripherally distributed pyknotic or karyorrhexic nuclei. The glomeruli appeared as more or less homogeneous, pink, spherical masses containing irregularly dispersed pyknotic and karyorrhexic nuclei and occasionally the ghosts of erythrocytes. The basement membranes of the glomerular tufts, and those of Bowman's capsule and the tubules were swollen, acidophilic, and occasionally fragmented. The small and medium-sized arterioles and venules within the necrotic cortices were swollen, eosinophilic, and devoid of recognizable cells. These vessels contained small numbers of red and white blood cells and small amounts of colloid material. They were devoid of thrombi, as were the glomerular and peritubular capillaries.

Situated in the juxtamedullary area was a band of inflammatory tissue 100 to 150 μ in thickness in which polymorphonuclear cells, some in advanced necrobiosis, were crowded together. These cells were regularly within the interstitial spaces between the tubules; they had nowhere crossed the basement membranes. The tubules within this narrow zone were necrotic, but they differed in an interesting way from the necrotic tubules in the outer cortex (as yet uninfiltrated with inflammatory cells), for the conglomerate remains of the cytoplasm of the tubular cells were stained even more deeply with eosin, while the remnants of the pyknotic nuclei had disappeared entirely—the further alterations depending, it seemed probable, on the action of enzymes liberated from the leukocytes.

The majority of the glomeruli in the juxtamedullary region were completely necrotic and resembled those of the cortex. Some, however, displayed a few unaltered capillary loops containing well preserved red blood cells and increased numbers of polymorphonuclear leukocytes. A few glomeruli in this region appeared normal and adjacent tubules and supporting structures likewise were unchanged. These small foci of viable tissue contrasted sharply with the more numerous and larger necrotic foci adjacent to them.

The interlobular arteries and veins appeared normal. Some segments of the arcuate and intralobular arteries likewise were normal, but wherever these vessels passed through the zone of inflammatory cells their walls abruptly became swollen and eosinophilic and obviously necrotic. Again thrombi were absent from all renal vessels.

Rabbit 12–43. Rabbit 12–43 died approximately 24 hours after injection of the toxin. The findings were like those in rabbit 13 except for an additional band of inflammatory cells (chiefly polymorphonuclear leukocytes) extending centrally from the renal capsule. Engorgement of the medullary vessels was extreme and occasional linear hemorrhages were seen.

Rabbit 61. Rabbit 61 died approximately 36 hours after injection of the toxin. The medulla was greatly engorged. With a band of inflammatory cells extending

centrally from the capsule and another peripherally from the juxtamedullary region, the findings resembled those in rabbit 12–43. Approximately half the juxtamedullary glomeruli appeared viable histologically, however, and the foci of viable tubular tissue in this region and in the pyramids were correspondingly increased in number and size.

Rabbit 71. Rabbit 71 died approximately 36 hours after injection of the toxin. Findings were like those in rabbit 12–43.

Rabbits 78 and 79. Rabbits 78 and 79 died approximately 38 hours after injection of the toxin. Marked hyperemia was present. Findings were like those in rabbit 12–43.

The abdominal viscera other than the kidneys were unchanged in all except one of the animals manifesting bilateral renal necrosis and so, too, were the thoracic viscera, these findings providing further evidence that the renal ischemia was highly selective. In the exceptional animal, bilateral infarcts of the adrenal glands, resembling those encountered by De Navasquez[8] under similar experimental conditions, were found. In this connection it may be noted that Doniach and Walker[18] found necrosis of the anterior pituitary gland in association with post-partum renal necrosis in 3 human beings. To learn whether this lesion might be present also in animals given staphylococcal toxin, the pituitary glands of the 9 rabbits with extensive bilateral renal necrosis were studied. These were all unchanged.

In a number of examples of naturally occurring and experimentally produced bilateral renal necrosis heretofore reported,[1-10] necrosis of the cortices has been incomplete and the medullae have been largely spared, though in most cases the process has involved practically the entire cortex and variable proportions of the medulla as well. The cases here reported were of the latter sort. In every instance, many focal areas of the medulla in addition to practically the entire cortex were rendered necrotic; indeed, in only one instance was as much as half the medullary tissue left viable. It seems obvious that the disease process cannot be considered "cortical" in any strict or unqualified sense. More will be said later about its pathogenesis.

Return of Renal Blood Flow to Previously Ischemic Kidneys, with Necrosis and Regeneration of the Epithelium of Scattered Nephrons, Probably Consequent to Transitory Ischemia

The results of the India ink test made it plain that the renal blood flow had returned to normal in the 5 animals sacrificed during the period from 2 to 7 days following injection of the toxin, while microscopic studies provided evidence of the effects of transitory ischemia in each instance.

The kidneys of the 2 animals of group e (given India ink and killed 2 days after injection of the toxin) were obviously blackened, and upon microscopic examination the ink was seen to fill the small renal vessels uniformly. In both instances, in addition to a coarse granularity and vacuolation of the cytoplasm of the tubular epithelial cells throughout the kidneys, there were many small foci in which the epithelium of single nephrons or groups of nephrons scattered throughout the cortices was necrotic. Such areas of necrosis were comprised of more or less amorphous granular

acidophilic material which filled the tubular lumen and was bounded by what appeared to be a normal basement membrane. Apart from rare glomeruli that showed swelling of solitary capillary loops, sometimes with slight hemorrhage into Bowman's space, the glomeruli and blood vessels were unchanged; likewise the basement membranes of the glomerular tufts and those of the tubules appeared normal in the preparations examined, though special stains were not made with the aim of disclosing subtle alterations.

As already indicated, an abundance of India ink was present in all glomerular and peritubular capillaries as well as in the larger vessels throughout the kidneys of these animals. It seemed especially noteworthy that there was regularly an abundance of ink in the capillaries adjacent to the necrotic tubules in the cortices (Fig. 4), the findings showing definitely that blood flow had returned to normal at the time of sacrifice and that occlusion of the small vessels by thrombi had not taken place to account for the focal necrosis.

The renal blood flow was likewise normal in the 2 animals sacrificed 4 days after injection of the toxin (group f), and the minute blood vessels throughout the kidneys in each instance were uniformly filled with ink. There was evidence of antecedent focal tubular necrosis with subsequent epithelial regeneration in one of the animals, however; while in the other the epithelial cells of the proximal convoluted tubules were conspicuously vacuolated. The nephrons displaying regeneration were randomly distributed. In the one animal sacrificed on the 7th day (group g), the tubules of many nephrons contained acidophilic débris and were lined by flattened hyperchromatic cells, while others displayed focal areas of calcification with surrounding lymphocytic infiltration.

From the previous sections it seems manifest that the renal ischemia that followed the injection of staphylococcal toxin was transitory in some instances and prolonged for at least 4 to 6 hours in others, while the anatomical changes ranged from nil through a more or less inconspicuous granularity and vacuolation or a more widespread but still focal necrosis of tubular epithelium, to an extreme bilateral renal necrosis. The granularity and vacuolation and the widespread focal necrosis of tubular epithelium were precisely like the changes produced by several workers who have mechanically interfered with the flow of blood through the kidneys; identical changes were produced also in control experiments of the present work by clamping the renal artery of rabbits for periods of 80 to 120 minutes. The more extreme changes of bilateral renal necrosis are generally assumed to result from ischemia.[7,8] In the present work an early bilateral cortical necrosis was actually observed in an animal in which the experimental findings made it plain that renal ischemia had persisted for 5 to 6 hours. Hence it seems reasonable to suppose that renal ischemia probably had persisted for longer periods in the 8 animals that manifested more extreme changes of bilateral renal necrosis. The findings as a whole provide strong evidence that the character of the anatomical changes observed in the various experimental animals depended upon the extent and duration of the renal ischemia.

Discussion

The selective effect of the toxin on the renal blood flow was one of the most striking findings of the present work. While the toxin regularly induced complete renal ischemia under suitable experimental conditions, it had no obvious effect on the flow of blood elsewhere in the body, except in the one instance in which adrenal infarction was noted. This observation was by no means new, for the studies of previous workers, although not conclusive in this respect, had provided indication that staphylococcal toxin somehow acts selectively on the renal blood vessels.[1-6] The special liability of these vessels to the vasoconstrictive effects of epinephrine and vasopressin had likewise been known for many years,[14,15] and the ability of electric stimuli, applied to special areas of the cerebral cortex, to bring about a selective renal ischemia was reported simultaneously.[16]

The amount of staphylococcal toxin given in the present experiments was decided upon initially more or less empirically, but the choice proved fortunate. As a consequence, renal ischemia proved transitory in some rabbits and enduring in others, resulting in the wide spectrum of anatomical alterations which have been described. The findings provided evidence that the extent and duration of the renal ischemia determined the character of the anatomical changes.

In the experimental studies on renal ischemia produced by vasopressin or by electric stimulation, and referred to previously, the flow of blood to the cortex was often curtailed while that through the juxtamedullary glomeruli and pyramids persisted apparently unaltered. The findings show that the blood vessels supplying the nephrons of the outer cortex differ from those supplying the juxtamedullary units, not only anatomically, as had previously been shown,[14] but also in the sensitivity of their responses to both neural and humoral stimulation. The functional difference had also been noted in the experiments of Trueta *et al.*[5,6] with staphylococcal toxin, and it was manifest in the present work. The point deserves emphasis, however, that in the present experiments partial renal ischemia was observed in relatively few of the animals studied, and, indeed, only in animals sacrificed either at the onset of the ischemic process or as the blood flow returned. Furthermore, the findings in the instances of partial ischemia showed that the blood vessels of nephrons in the outer cortices did not react uniformly in one way while those of the juxtamedullary nephrons reacted uniformly otherwise. In certain instances the India ink tests showed that many radially arranged segments of the cortices had functioning vascular channels while other segments were ischemic;

furthermore, in other instances, while the tests showed that flow of blood through the juxtamedullary glomeruli persisted to some extent and was greater than that through the outer cortices, still it was greatly restricted, and half or more of the juxtamedullary glomeruli in these instances contained stagnant blood which prevented perfusion with India ink. Taken together, the findings indicate that the amount of staphylococcal toxin given and the duration of its effect, as well as individual variations in susceptibility to it, were singly or in the aggregate more influential in determining the course of events than were anatomical and physiologic differences in the blood vessels of the cortical and juxtamedullary glomeruli.

The findings in the instances of partial ischemia had a more important implication. The partial ischemia induced by staphylococcal toxin was remarkably similar to that observed in animals given vasopressin or electric stimuli to the cerebral cortex, as already mentioned; and since good evidence indicates that renal vasospasm can be held responsible for the ischemia induced by the two latter means, it seems reasonable to suppose that it might also be responsible for the renal ischemia brought about by staphylococcal toxin. Four additional observations made during the course of the present work bring the possibility nearer. (1) The renal veins of the animals given staphylococcal toxin were examined at laparotomy prior to and following the injection of India ink into the aorta; during the period 40 to 120 minutes following injection of the toxin they were seen regularly to be in spasm or collapse. (2) The small blood vessels of the ischemic kidneys were greatly distended with blood, as histologic studies showed, the anatomical findings providing evidence that the columns of blood in the small vessels were stagnant, perhaps owing to spasm of the venules or veins, thus bringing about ischemia and preventing the entrance of India ink under the test conditions. (3) Thrombi were absent from the blood vessels of the ischemic kidneys, as careful microscopic studies showed; while (4) the presence of India ink (indicating the free flow of blood under the test conditions) in peritubular capillaries adjacent to foci of tubular necrosis or regeneration in animals killed several days after injection of the toxin (Fig. 4) showed that blood flow had returned in areas of parenchyma that had previously been ischemic. No attempt was made to learn whether the effect of staphylococcal toxin on blood flow was mediated through the autonomic nerves. Yet the findings as a whole provided strong evidence that spasm of the renal blood vessels, perhaps beginning initially in the veins or venules, was responsible for the ischemia and thus indirectly for the anatomical changes consequent upon it.

The probability that vasospasm brought about ischemia and that this in turn gave rise to the anatomical changes observed in the present work has implications for the pathogenesis of bilateral renal necrosis, for this process was observed in 10 of the rabbits given staphylococcal toxin. While numerous observations have made it plain that the anatomical changes in both the naturally occurring disease and in its experimental counterpart are almost surely the result of partial or nearly complete ischemia, the cause of the ischemia has remained obscure.[7-10,14] True, in a number of instances thrombi have been found in renal blood vessels of human beings with bilateral cortical necrosis of the kidneys, though whether they caused the ischemia or resulted from it could not be decided.[7,8,10] Furthermore, from the recent observation of Thomas and Good[17] that thrombi giving the staining reactions of fibrin are regularly present in the glomerular capillaries and other small renal blood vessels of rabbits exhibiting renal necrosis as a manifestation of a generalized Shwartzman reaction, it seems obvious that capillary thrombi, or intracapillary precipitates like those recently encountered by Hausman and Dreyfus[18] in rabbits given sodium polyanetholene, might give rise under suitable conditions to renal necrosis resembling that seen in the naturally occurring disease. Yet the point deserves emphasis that thrombi have been inconspicuous in, or absent from, the renal blood vessels in most of the naturally occurring cases of renal necrosis thus far studied.[7-10] Furthermore, they were not found in previously studied instances of renal cortical necrosis complicating the generalized Shwartzman reaction,[19] and they were not encountered in the disease produced experimentally with staphylococcal toxin, either in the present work or in that of previous investigators.[1-6] Their absence under these diverse circumstances strengthens the probability that renal ischemia and necrosis often result, at least under some experimental circumstances and perhaps under natural conditions also, from widespread renal vasospasm.

The findings of the present work have additional interest in relation to the recent studies of Oliver, MacDowell, and Tracy,[20] which indicate, in essence, that ischemia is responsible for the focal tubular changes and consequent alterations in renal function that are seen in some cases of shock and in other disease states characterized in part by acute anuria. It is plain, however, that the ischemic changes observed by these authors were quite different from those noted in the present work; for, although numerous and widespread, the ischemic lesions which they described were not only focal but extremely minute.

Their findings, in concert with those of Schlegel and Moses,[21] suggest that localized segments of the peritubular capillaries became devoid of blood, perhaps as a result of hypotension and collapse or spasm, with result in death of small numbers of cells and fragmentation of the basement membrane underneath them in minute focal segments of the tubules. In the ischemic kidneys of rabbits given staphylococcal toxin, by contrast, a wide range of anatomical changes was produced, and even when the changes were focal they involved the bulk of the tubular epithelium in a given nephron as if the blood supply to the entire unit had been shut off. Obviously, however, much remains to be learned about the state and functioning of the small renal vessels under normal and abnormal conditions before the different manifestations of ischemia can be fully comprehended.

Summary

To learn more about the effects of a bacterial toxin on the kidney, and in particular about the implications of these effects for the pathogenesis of the disease process known as symmetric renal cortical necrosis, a systematic study was undertaken of the functional and structural changes that follow the injection of staphylococcal toxin intravenously in rabbits.

A standardized amount of toxin, formed by the Wood strain of *Staphylococcus aureus* grown on Leonard-Holme medium, when given intravenously to adult rabbits, regularly produced within 15 to 20 minutes a more or less complete spasm or collapse of the renal veins, as was observed directly in the living animal. Furthermore, when India ink was injected into the aortas of rabbits during the period 40 to 120 minutes following injection of the toxin, it regularly failed to enter the blood vessels of the kidneys but freely perfused those of the remaining abdominal viscera and the lower limbs. Yet the kidneys of each rabbit examined during the period 40 to 120 minutes were turgid and cyanotic, and microscopic examination showed that most of their small blood vessels were greatly distended with blood. The findings were interpreted as indicating a virtually complete renal ischemia resulting from a selective effect of the staphylococcal toxin on the renal blood vessels, the initial disturbance being probably a spasm of the venules or veins, resulting in an extreme "stagnation" hyperemia and functional ischemia. No such effects were produced by the toxin when it had been rendered non-hemolytic for rabbits' erythrocytes and nonlethal for mice by procedures such as heating at 60° C. for 30 minutes or admixture with specific antitoxin *in vitro*.

The stagnation hyperemia and functional ischemia persisted in approximately half the rabbits given the India ink test during the period of 3 to 6 hours after the toxin had been injected, while in the rest the findings provided evidence that the blood flow had returned to normal. Microscopic studies disclosed varying degrees of congestion in the kidneys of the animals studied during this interval, together with a vacuolation and increased granularity of the cytoplasm of the epithelial cells of the convoluted tubules in the animals in which the ischemia had been prolonged. The latter changes were interpreted as being minor and perhaps reversible cellular effects of ischemia of comparatively short duration.

Somewhat more than half the rabbits that had first been given staphylococcal toxin and then were allowed to survive 6 hours or more, died during the interval from 9 to 38 hours. The kidneys of the 10 animals of this group all manifested varying degrees of necrosis, the lesions being quite similar both in character and in extent to those seen in naturally occurring symmetric cortical necrosis of the kidneys in human beings. The fact was noteworthy that thrombi were absent from the renal blood vessels in all the instances studied.

In animals examined 2 to 7 days following injection of the staphylococcal toxin the results of the India ink test made it plain that the flow of blood in virtually all renal vessels was normal. Yet signs of necrosis of the epithelial cells of numerous nephrons scattered throughout the cortices were regularly found upon microscopic study, and in several instances manifestations of regeneration of tubular epithelium were noted. The finding of peritubular capillaries filled with India ink alongside necrotic or regenerating tubular epithelium in animals that had been given toxin several days prior to the application of the India ink test, provided clear evidence that blood vessels in areas that had previously been ischemic were once more patent and functioning.

The observations as a whole suggest that the anatomical changes which became manifest in the kidneys of the rabbits given staphylococcal toxin depended largely upon the extent and duration of the renal vasospasm and ischemia. They provide an example of the special reactivity of the renal vascular system to a circulating toxin, and give further evidence that symmetric cortical necrosis may result from ischemia induced by prolonged spasm of the blood vessels.

The findings were discussed in relation to those seen in other disease states characterized by acute renal ischemia and anuria.

The photographs were made by Mr. Julius Mesiar.

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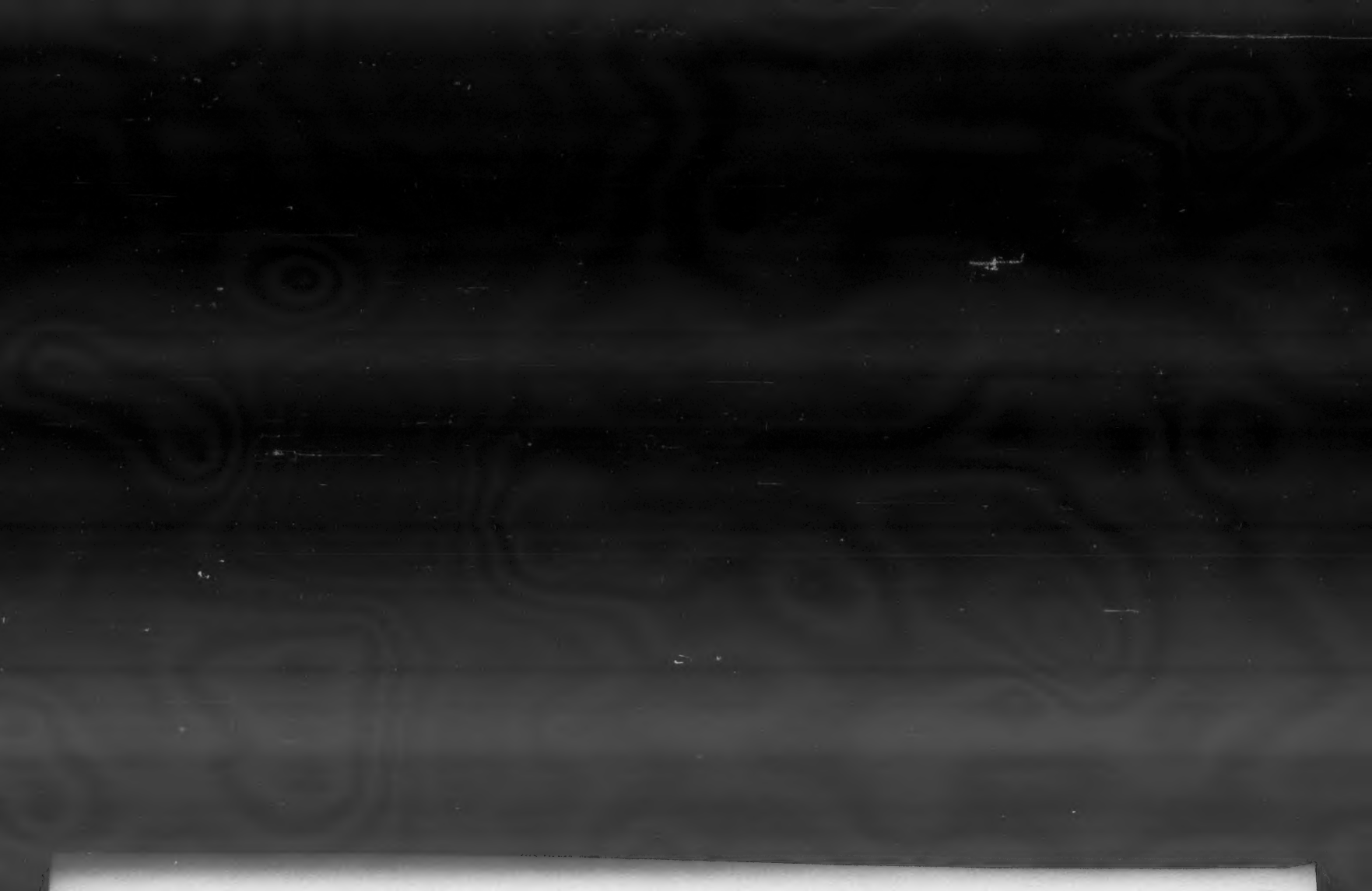
LEGENDS FOR FIGURES

FIG. 1. Abdominal viscera of a rabbit that had been injected first with staphylococcal toxin in a marginal ear vein and then, 2 hours later, with India ink in the abdominal aorta. Five minutes after injection of the ink the animal was killed and photographed. The liver, spleen, stomach, and intestines were all more or less regularly blackened by the ink in their blood vessels. The right kidney, by contrast, failed entirely to blacken, as did also the left (not shown). For comparison with Figures 2 and 3.

FIG. 2. For comparison with Figure 1. A control rabbit was given 0.9 per cent NaCl in a marginal ear vein, and 2 hours later India ink into the abdominal aorta. The kidneys and other viscera blackened concurrently, the process beginning immediately after injection of the India ink had been started.

FIG. 3. On the left are shown the kidneys of 2 control rabbits given 0.9 per cent NaCl solution into an ear vein and, 2 hours later, India ink into the aorta. On the right are shown the kidneys of 2 rabbits that had been given staphylococcal toxin in an ear vein and, 2 hours later, India ink into the aorta. The findings are representative of those in 10 control and 8 experimental rabbits of a single experiment. When first removed, the kidneys of the rabbits given staphylococcal toxin were deeply cyanotic; they became pink during the interval before the photograph was made.



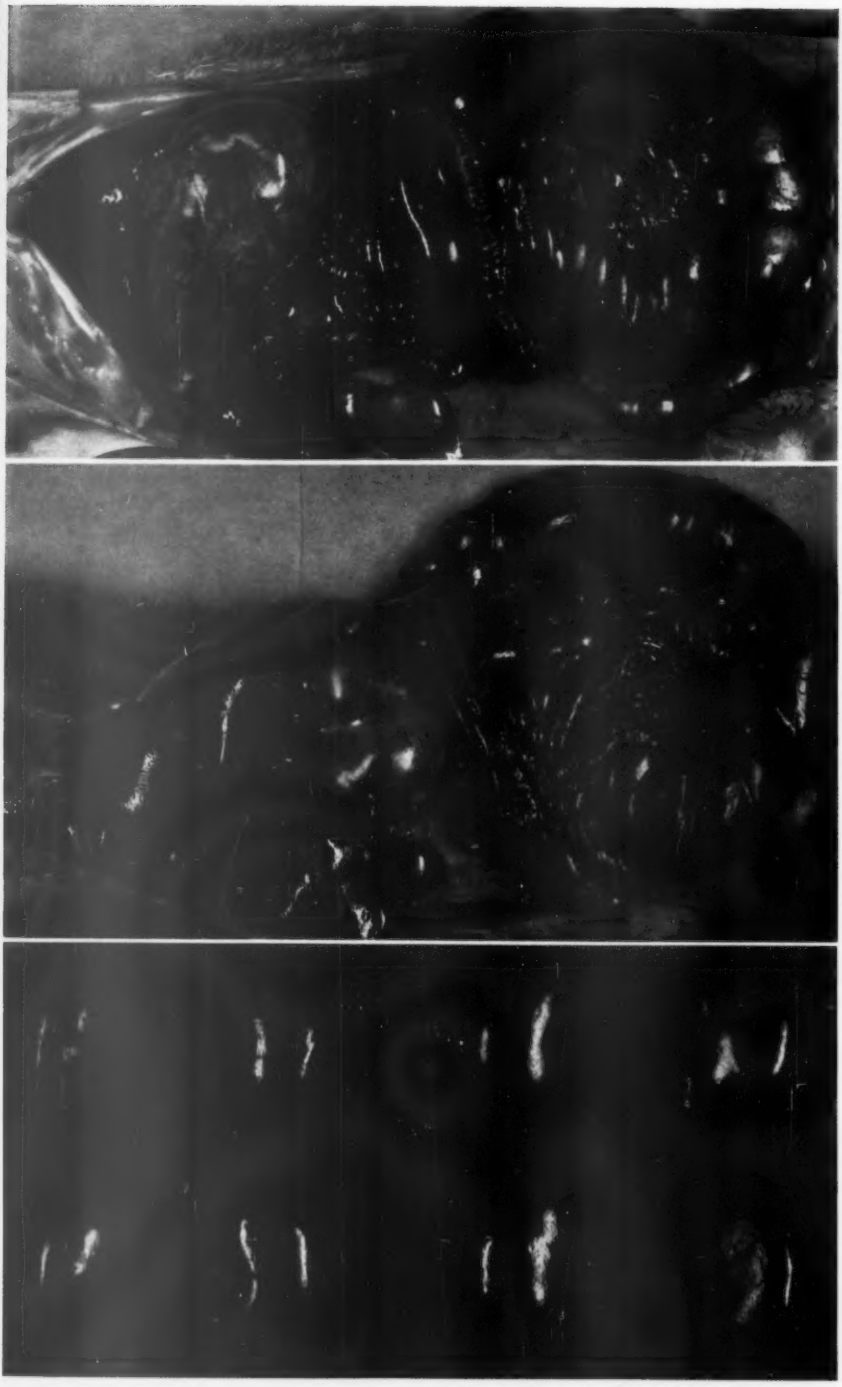

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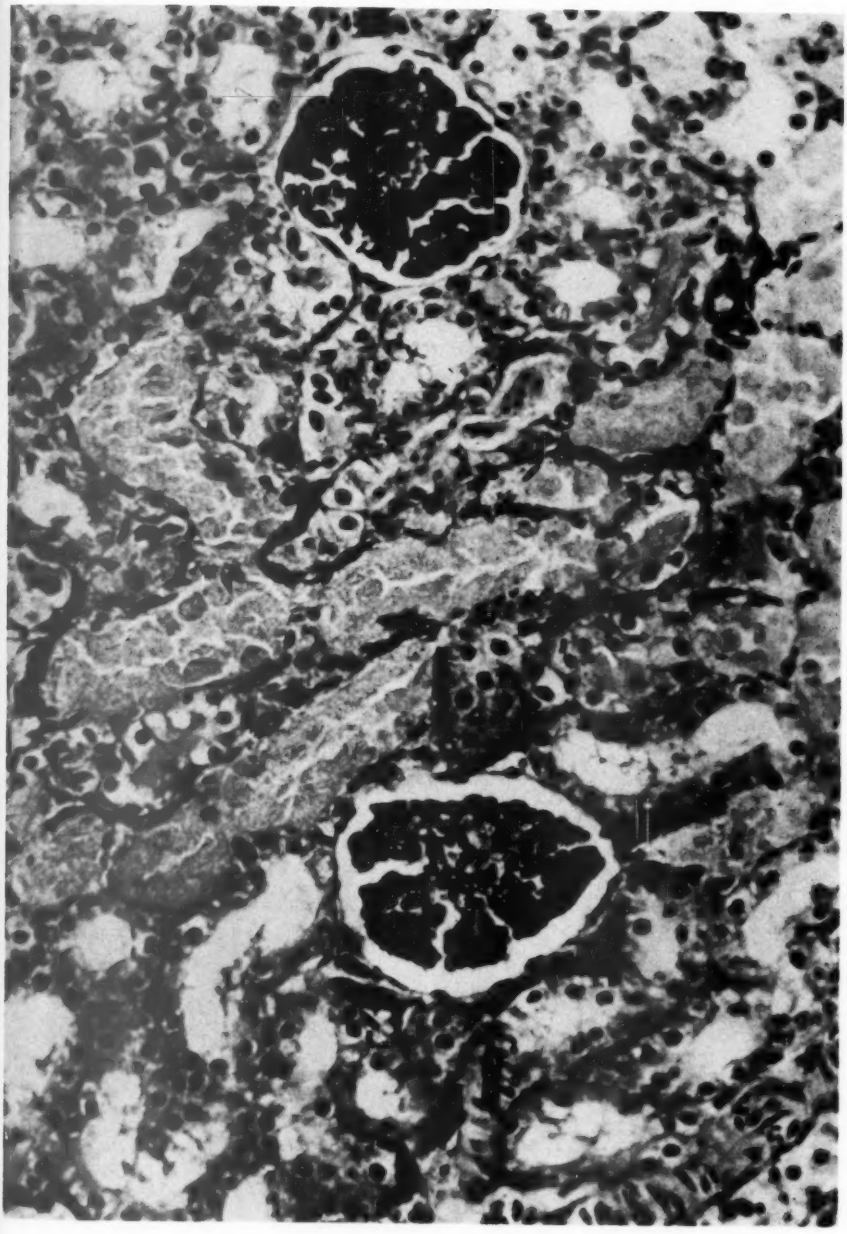
FIG. 4. Histologic changes in the renal cortex of an animal given staphylococcal toxin intravenously into a marginal ear vein and then, after an interval of 48 hours, India ink into its abdominal aorta. Focal necrosis of the tubular epithelium is obvious, as is also perfusion of the adjacent glomerular and peritubular capillaries with ink. The results provide evidence that the blood flow had returned to areas of the kidney that were previously ischemic. Approximately × 360.



4

UREMIC PNEUMONITIS *

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Systemic manifestations of uremia are classified ordinarily under headings indicating involvement of the central nervous, gastrointestinal, respiratory, and cardiovascular apparatus. Necrotizing and inflammatory reactions have been described in the gastrointestinal tract, heart, pericardium, pleura, peritoneum, spleen, skin, and synovia, and have been related to the signs and symptoms of uremia. Many of the necrotizing and inflammatory lesions which characterize uremia have been described in detail, especially those of the heart, pericardium, gastrointestinal tract, and skin. Various pathologic changes in the respiratory system have been reported. Fishberg[1] mentioned bronchitis and, more recently, Allen[2] has described an interstitial pneumonitis. Doniach[3] and Bass and Singer[4] have summarized the clinical, roentgenographic, and pathologic pulmonary findings in several cases of uremia. However, few descriptions of characteristic pulmonary lesions have appeared in the literature.

Approximately 12 years ago we became aware of pulmonary changes associated with uremia and, since that time, we have studied the lungs of 107 individuals who died with uremia, and have made comparable studies in a control group of 429 individuals who did not have uremia at the time of death. From this we have concluded that morphologic changes in the lungs of many persons with uremia (62 per cent in this series) are sufficiently distinctive to warrant application of the term *uremic pneumonitis*.

Gross Characteristics

Gross characteristics of the lungs in uncomplicated uremic pneumonitis include moderate increase in weight and diffuse rubbery induration. Often these lungs did not collapse to the usual extent when the thoracic cavity was opened. Cut surfaces were pale red and usually drier than anticipated from the increased weight and diffuse induration. In a few cases the limitation of the process to the more central areas of the lungs was appreciated and described. Hyperemia and edema were variable, probably related to congestive failure as a ter-

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minal complication. Fibrinous pleuritis was frequent, being present in approximately 20 per cent of cases. Of the 66 cases of uremic pneumonitis studied, 25, or approximately one third, were uncomplicated by bacterial pneumonia, infarction, or chronic passive congestion. The average weight of the lungs (together) in this group was 1,008 gm.

Microscopic Characteristics

Microscopically, lungs with uremic pneumonitis exhibited slight to moderate edema, usually rich in protein and with amorphous or granular protein precipitate evident. More striking was the considerable amount of fibrin present in the alveoli. This was arranged as a fine network (Figs. 1 and 2), occasionally in dense hyaline masses or bands (Figs. 3 and 4), and sometimes adherent to the alveolar wall in the form of an asphyxial membrane (Figs. 5 and 6). Frequently there were small numbers of erythrocytes present in this fibrin-rich edema fluid, apparently representing hemorrhage *per diapedesin*. Granulocytes were absent in cases uncomplicated by bronchopneumonia or other septic process. Cellular "exudate" usually was evident, however, made up principally of macrophages.

In uncomplicated cases there was no evidence of necrosis of alveolar septa or of thrombosis. Acute passive congestion, when present, involved principally arteries and veins, tending to spare alveolar capillaries. Interstitial pneumonitis was observed only occasionally. Bronchiolitis, obliterative or otherwise, was not a prominent feature. Necrotizing arteritis, suggested as a possible factor in pathogenesis of these lesions by Bass and Singer,[4] was not observed in our series. All sections studied were embedded by the celloidin technique. The routine staining procedure employed hematoxylin and eosin. Special staining procedures used in selected cases included van Gieson's, Gram-Weigert's, azocarmine, phosphotungstic acid hematoxylin, and periodic acid-Schiff's stains, and silver impregnation for reticulin.

Incidence

Correlation with Extrapulmonic Manifestations of Uremia. One hundred and seven cases of uremia, with material adequate for this study, were collected from the University of Chicago necropsy files covering a period of 40 years (1902–1942). From microscopic examination of the lungs a diagnosis of uremic pneumonitis was made in 66 cases, an incidence of 62 per cent. Of the 107 cases of uremia, 60 (56 per cent) had uremic pericarditis and 26 (24 per cent) had either gross or microscopic evidence of uremic colitis.

Of the 66 cases with uremic pneumonitis, 33 (50 per cent) had pericarditis also and 13 (20 per cent) had uremic colitis also. In only 8 (7 per cent) of the 107 cases of uremia studied, did uremic pneumonitis, pericarditis, and colitis occur together.

The diagnosis, uremic pneumonitis, was graded on the basis of severity and extent as 1 plus to 4 plus. Nine of the 66 cases were diagnosed 4 plus (14 per cent); 20 cases, 3 plus (30 per cent); 19 cases, 2 plus (29 per cent); and 18 cases, 1 plus (27 per cent).

Correlation with Other Changes in the Lung. Of the 66 cases of uremic pneumonitis, 38 exhibited evidence of coexisting bronchopneumonia either grossly or microscopically, while 28 (42 per cent) were not associated with bacterial pneumonia. In 4 cases there was chronic passive congestion; in 3 there were infarcts and/or thrombi. In 3 cases purulent bronchitis was present without significant bronchopneumonia. Atelectasis was present in nearly half of the cases, but usually was marginal or limited to small foci elsewhere.

Evaluation of Unknowns

To test the validity of our concept and to evaluate the histopathologic criteria of uremic pneumonitis just set forth, sections of the lungs from 461 consecutive necropsies (1049 sections) were reviewed. These were evaluated as unknowns and any diagnosis of uremic pneumonitis was made without knowledge of clinical data or gross necropsy findings and entirely upon the microscopic appearance of the lungs. In 44 cases, uremic pneumonitis was diagnosed on this basis. Of these, the diagnosis of uremia had been established in 27 cases (61 per cent). In 8 additional cases (18 per cent) there was at least moderately severe renal disease or clinical data which made the presence of uremia likely. In 9 cases (21 per cent), histologically diagnosed as uremic pneumonitis, there was no evidence of uremia clinically or from necropsy findings. Further study of these 9 cases revealed the following pertinent diagnoses: resolving lobar pneumonia (2 cases); organizing pneumonia; asthma with arteriosclerotic heart disease; carcinoma of lung with organizing and resolving pneumonia (2 cases); carcinoma of hypopharynx with atypical pulmonary congestion and edema; carcinoma of stomach with necrotic pulmonary metastases; carcinoma of pancreas and pernicious anemia.

Pathogenesis

Pathogenesis of the uremic syndrome is not clear. There is abundant evidence that retention of nitrogenous products such as urea, creati-

nine, and uric acid are not responsible for signs, symptoms, and pathologic changes. Retention of phenols, guanidine, indol, or unknown toxic substances has been considered by some to be most important. Volhard[5] has pointed out that the xanthoproteic reaction most closely parallels uremia and that this measures aromatic substances in general, including phenols (mostly conjugated), dihydroxy phenols, and oxy- acids. Bradley,[6] in his review of the pathologic physiology of uremia, stated: "The derangement of total chemical structure of the plasma, convincingly shown to be present in every case, seems to provide an adequate explanation for the impressive array of symptoms."

Explanation has been offered for the development of various inflammatory and necrotizing lesions which occur in relation to uremia, but in no case is the explanation entirely acceptable. For instance, it has been suggested that uremic colitis, often ulcerative or membranous, is the result of bacterial conversion of increased amounts of urea to ammonia. Some of the dermatologic manifestations of uremia (uremides) have been attributed to very high local concentrations of urea. It has been suggested that focal myocardial necrosis is an effect of hypocalcemia. No satisfactory pathogenesis has been offered for the pericarditis, which is one of the more common inflammatory reactions resulting from uremia.

Clinical Pathologic Correlation

As a basis for considering the pathogenesis of uremic pneumonitis, the 107 cases of uremia studied were evaluated from the standpoint of age and sex of the patient, etiology of the uremia, duration of uremia, blood chemical changes, including blood urea nitrogen, nonprotein nitrogen, serum chlorides, serum pH and CO_2-combining power, blood pressure, and whether oxygen therapy had been used. Table I summarizes this data.

From this evaluation it appears that age and sex do not vary significantly in patients with uremic pneumonitis as compared with the total group, nor is there apparent difference dependent upon the type of renal disease underlying the uremia. Concerning the duration of uremia, the data are not very reliable in that group in which signs or symptoms were evident for less than 1 month before death, since many of these patients were unable to give a reliable account of their "present illness." With this consideration, there appears to be no significant correlation between duration of uremia and occurrence of uremic pneumonitis. It is evident that uremic pneumonitis may develop

quickly (several days) after onset of uremia and that it may be present in patients who have had uremia for longer than 6 months.

Regarding chemical changes of the blood, uremic pneumonitis did

TABLE I

Data Pertaining to the Pathogenesis of Uremic Pneumonitis

Attribute and number of cases			With uremic pneumonitis	Without uremic pneumonitis
Sex	Male 77		44 (57%)	33
	Female 30		22 (73%)	8
Age	Average 45.2 years		43.6 years (average)	48.1 years (average)
Etiology	Glomerulonephritis	35	23 (66%)	12
	Pyelonephritis	25	12 (48%)	13
	Arteriolonephrosclerosis	20	14 (70%)	6
	Hydronephrosis	10	6 (60%)	4
	Amyloidosis	6	4	2
	Polycystic kidneys	4	2	2
	Cholemic nephrosis	3	2	1
Duration (of uremia)	Less than 1 week	19	16 (84%)	3
	1 week to 1 month	31	20 (65%)	11
	1 month to 6 months	32	18 (56%)	14
	More than 6 months	4	4	0
	B.U.N. or N.P.N. (mg. per cent)			
Azotemia (highest terminal value)	15–30 30–60	5	0	5
	30–60 60–120	14	4 (28%)	10
	60–120 120–240	39	31 (79%)	8
	120–240 240–480	18	15 (83%)	3
Serum Cl, mEq/l (lowest, terminally)	80–100	12	8	4
	100–110	5	4	1
	110–120	4	3	1
Serum CO_2 comb. p., mEq/l (lowest, terminally)	5–10	8	8	0
	10–15	9	7	2
	15–20	10	8	2
	20+	5	3	2
Blood pressure, systolic (highest value)	120–160 (mm. of Hg)	36	26 (72%)	10
	160–200	27	15 (55%)	12
	200+	28	22 (79%)	6
Blood pressure, diastolic	80–90 (mm. of Hg)	31	22 (71%)	9
	91–100	11	6	5
	101–110	7	4	3
	110+	41	30 (73%)	11
Oxygen therapy	Yes	8	6 (75%)	2
	No	25	17 (68%)	8

not occur in patients with blood urea nitrogen below 30 mg. per cent (or non-protein nitrogen below 60 mg. per cent). A high incidence of uremic pneumonitis did not occur until blood urea nitrogen values were over 60 mg. per cent (non-protein nitrogen over 120 mg. per

cent). Serum chloride levels were not related to the incidence of uremic pneumonitis.

Most significant was the fact that of 8 patients with serum CO_2-combining power below 10 mEq/l, all had uremic pneumonitis, and of 26 patients who had a CO_2-combining power below 20 mEq/l, 24 (92 per cent) had uremic pneumonitis.

Comment

Morphologic changes which characterize uremic pneumonitis have undoubtedly been observed by many pathologists. The relatively few recorded descriptions of this process in the lungs of uremic patients may be due to the fact that each of the component changes are, in themselves, quite non-specific. The process may be confused with resolving and organizing pneumonia, as is indicated by Doniach[3] and as is seen from the summary of missed diagnoses in our "unknown" cases in which 6 of 9 cases represented resolving or organizing pneumonitis of bacterial origin or reaction peripheral to neoplasm. Characteristic changes of uremic pneumonitis may be dismissed as protein-rich edema fluid or perhaps considered as a non-representative section taken near a focus of bacterial pneumonia. The diffuse nature and homogeneous appearance of uremic pneumonitis are best recognized in uncomplicated cases in which several sections of each lung are studied.

The diffuse pulmonary lesion which most closely resembles uremic pneumonitis is rheumatic pneumonitis. In our experience rheumatic pneumonitis usually is accompanied by more hemorrhage and by necrosis of alveolar septa.

That this uremic pulmonary lesion deserves wider recognition by pathologists is suggested by the number of papers in the literature of Roentgenology describing the characteristic pulmonary lesions. Furthermore, the condition sometimes is important in differential diagnosis. One of us recently performed a necropsy on a patient with malignant hypertension in whom uremic pneumonitis probably was responsible for the presenting symptoms, which led to difficulty in diagnosis since he was considered to have bacterial pneumonia.

Several observers have suggested that left heart failure is important in the pathogenesis of uremic pneumonitis and that elevation of pulmonary venous pressure plus an increase in capillary permeability, due to toxic products associated with azotemia, are responsible for the outpouring of fibrin into the alveolar spaces. Our studies indicate that the lesions may occur with little or no clinical or pathologic evidence

of left heart failure. Furthermore, it seems that the degree of acidosis, as judged by the depression in blood CO_2-combining power, is more readily correlated with the development of the lesions. The fact that uremic pneumonitis appears to occur less often in recent cases of uremia may reflect more effective control of acid-base balance in modern therapy of uremia.

SUMMARY

The gross and microscopic characteristics of uremic pneumonitis are described and correlated with other inflammatory and necrotizing manifestations of uremia.

The etiology and pathogenesis of uremic pneumonitis are discussed.

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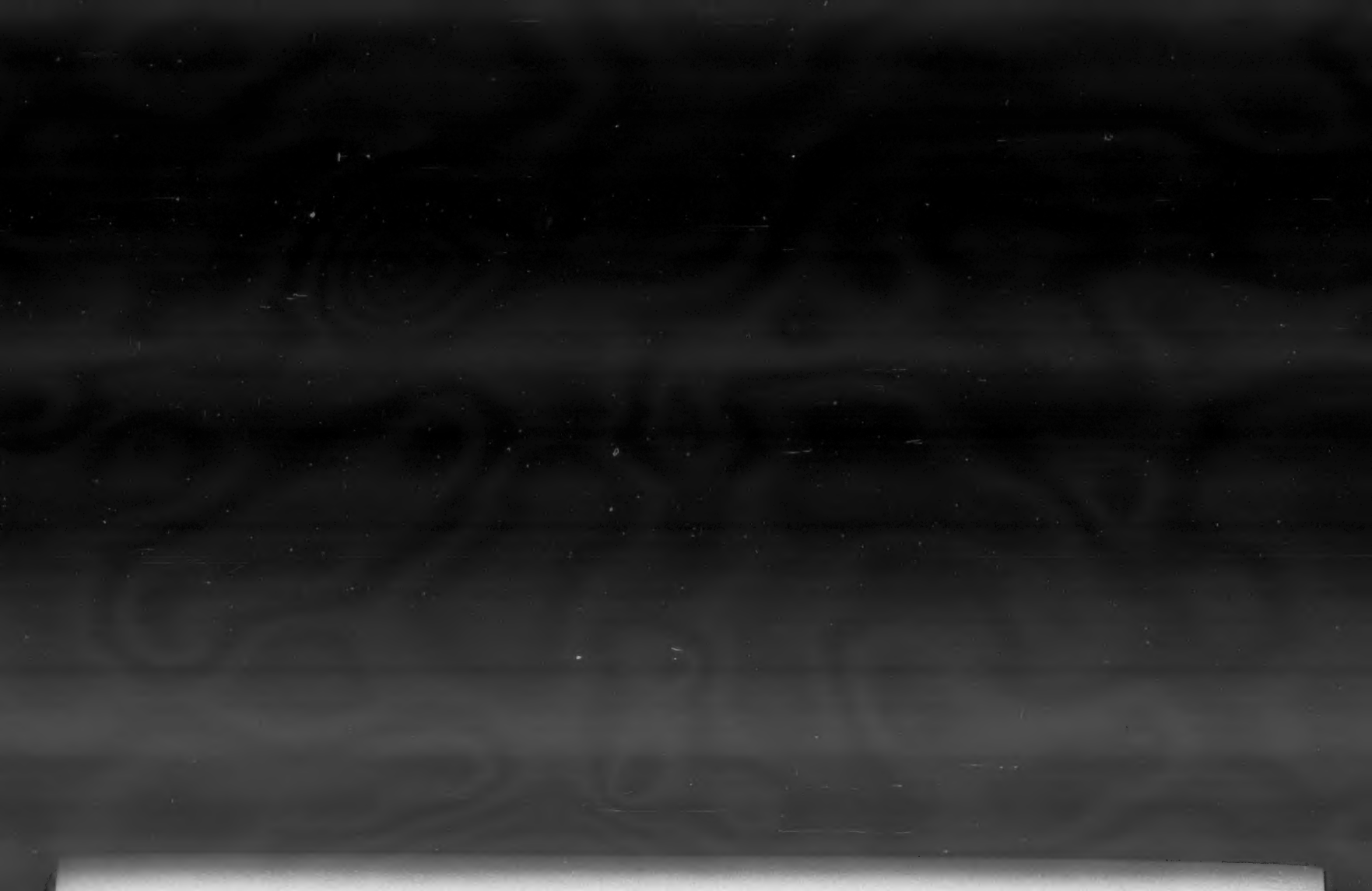
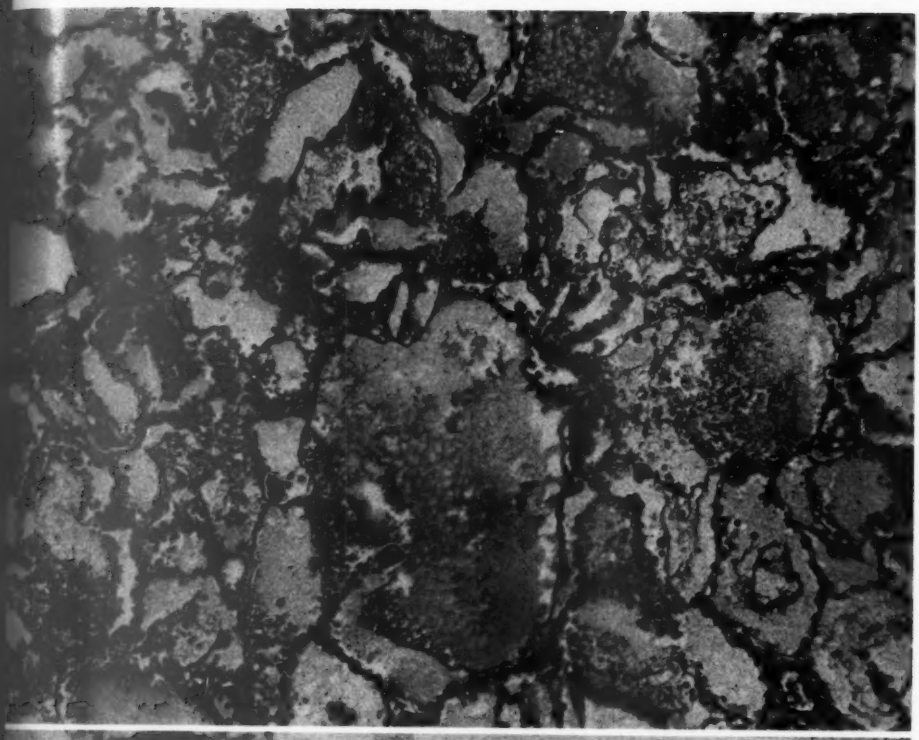
[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. A-3728. The protein-rich edema fluid contains a small amount of fibrin. Intra-alveolar cells are principally macrophages. Hematoxylin and eosin stain, celloidin embedded. × 125.

FIG. 2. A-3512. Fibrin is a prominent constituent of the edema fluid. Hematoxylin and eosin stain, celloidin embedded. × 165.





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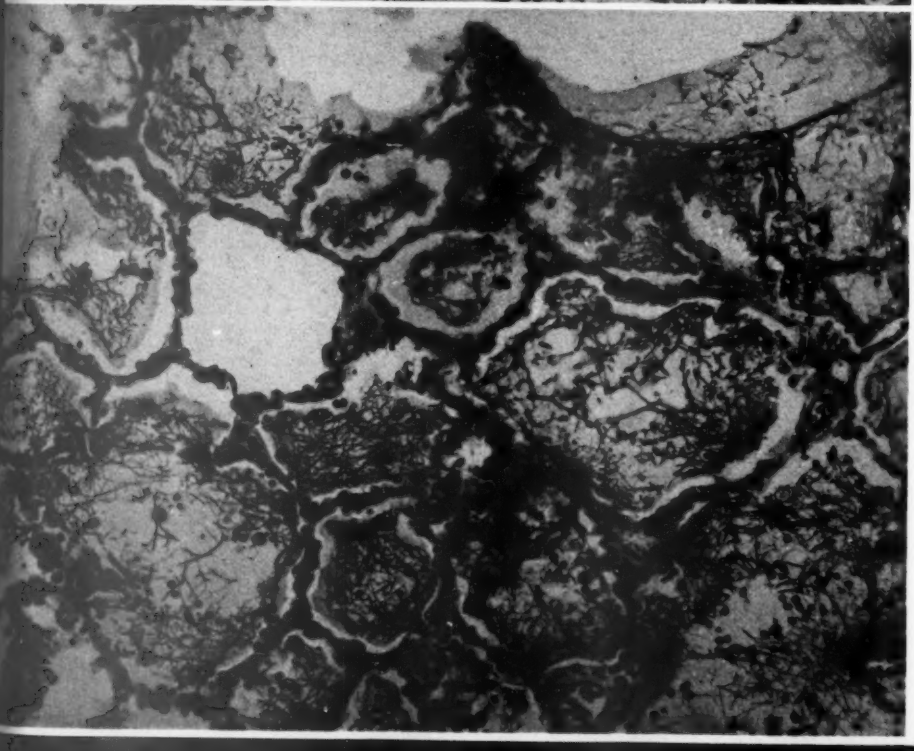

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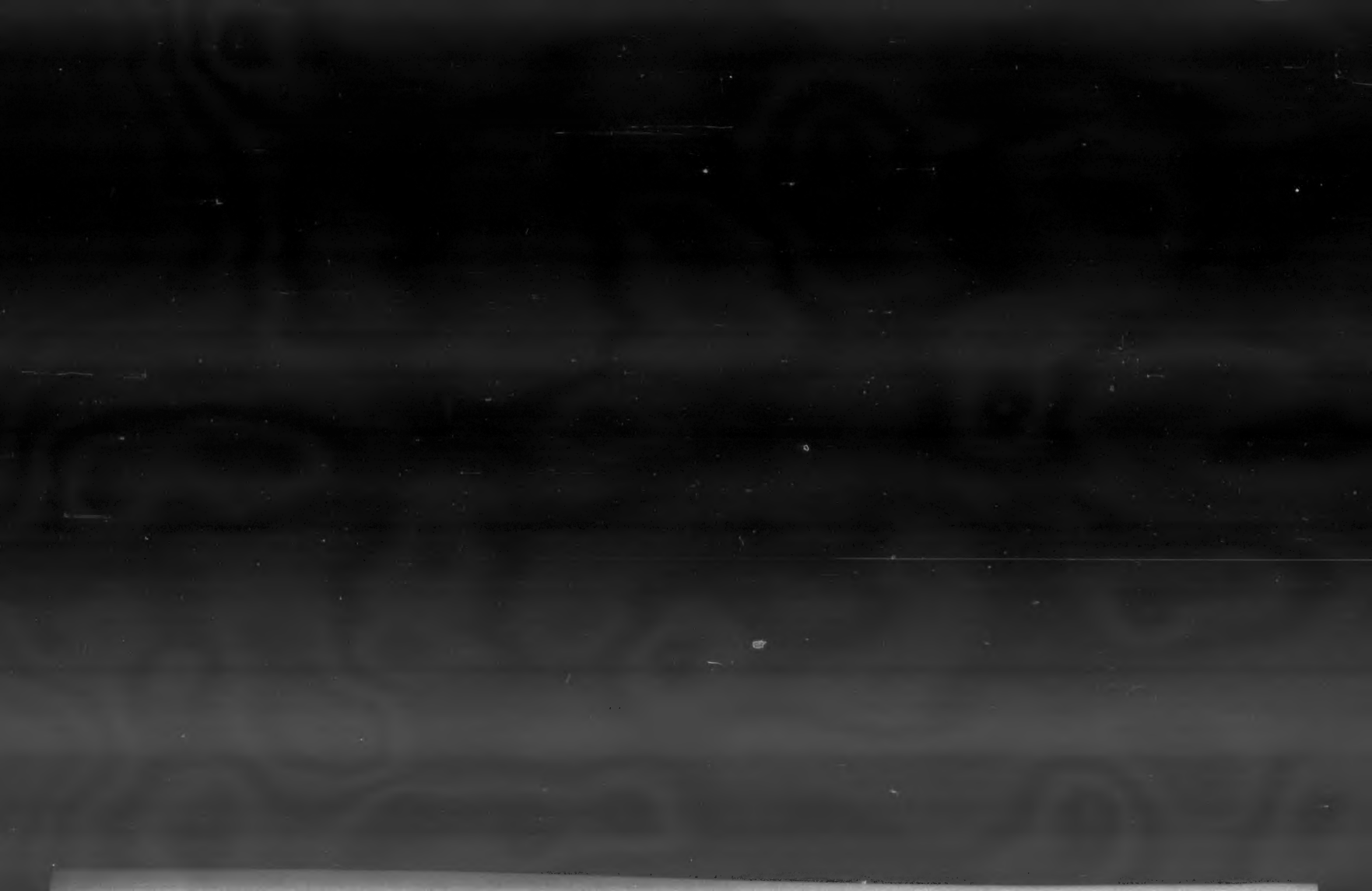
FIG. 3. A-4317. Fibrin is quite abundant and is massed in dense clumps. Cellular exudate is composed principally of macrophages. Hematoxylin and eosin stain, celloidin embedded. × 65.

FIG. 4. A-4317. Fibrin is quite abundant and is massed in dense clumps. Cellular exudate is composed principally of macrophages. Hematoxylin and eosin stain, celloidin embedded. × 165.



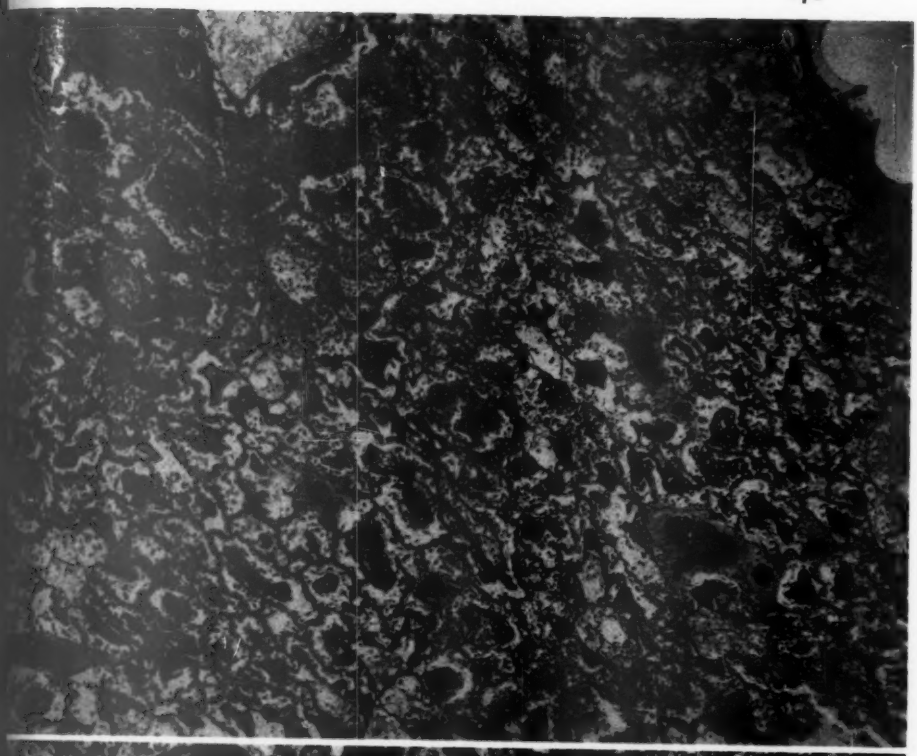


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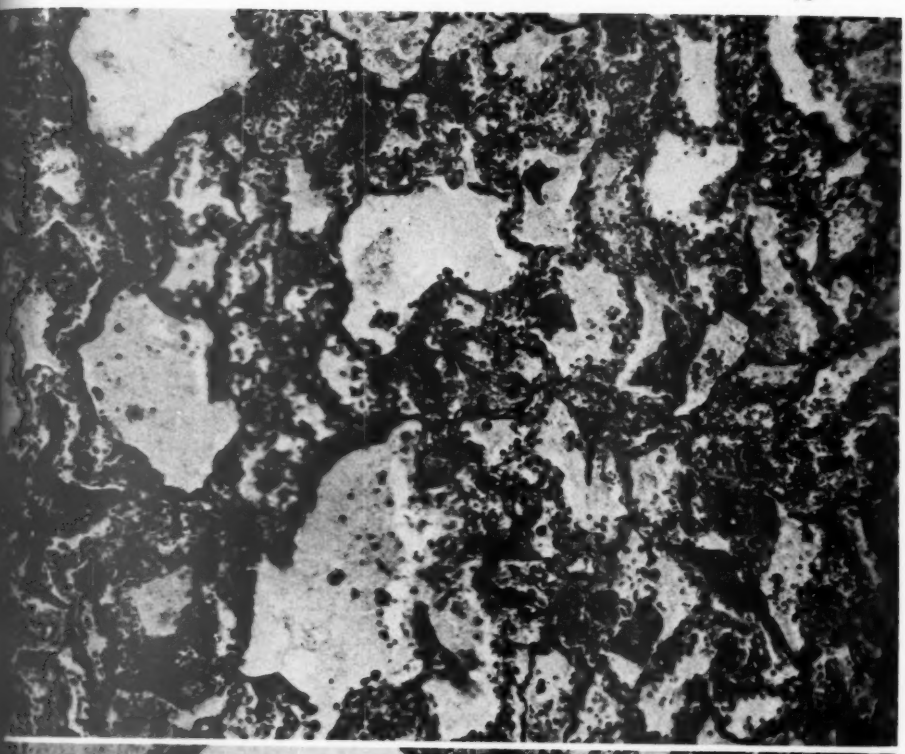
FIG. 5. A-4129. Fibrin is arranged in the form of an asphyxial membrane. Hematoxylin and eosin stain, celloidin embedded. × 125.

FIG. 6. A-3885. In addition to protein-rich edema fluid and fibrin in membranous form, there is focal atelectasis and emphysema. Hematoxylin and eosin stain, celloidin embedded. × 125.



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6

NODULAR AND FATTY GLOMERULAR LESIONS IN RABBITS ON CORTISONE *

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During the past few years discrete nodular lesions have been observed in the glomeruli of rabbits receiving cortisone by Rich, Berthrong, and Bennett[1]; Becker[2]; McLean, Hamilton, and Fitzgerald[3]; Germuth,[4] and Bloodworth and Hamwi.[5] These lesions are said to resemble those of diabetic glomerulosclerosis in man. Cortisone administration has not been shown to produce similar lesions in other species, including man. ACTH apparently does not cause similar glomerular changes in rabbits unless, according to Becker, they have been previously rendered diabetic with alloxan.

One of the more distinctive features of the reactions of rabbits to cortisone administration is the rapid development of lipemia[6,7] that persists for many weeks when the drug is continued.[8] This lipemia resembles alimentary lipemia in that the most striking increase is neither in the cholesterol nor phospholipid fractions.[8] Gordon, Kobernick, McMillan, and Duff[9] noted an increase of the fatty acids of neutral fats. Cortisone administration in other species does not produce comparable degrees of lipemia. Administration of ACTH to rabbits usually does not cause grossly visible lipemia.[6] Since the glomerular lesion and lipemia represent two peculiar reactions of the rabbit to cortisone administration, the question arises as to whether these two phenomena are related.

Most observers who have described the lesions of Kimmelstiel-Wilson's disease have noted the occasional presence of lipid masses in the involved tufts. In a previous study[10] it was found that lipid deposits occur with greater frequency and severity in Kimmelstiel-Wilson's disease than in any other form of renal disease. It was suggested that these lipid deposits may play an important rôle in the development of the lesions. Bloodworth and Hamwi[5] mentioned lipid deposits in the experimental lesions in cortisone-treated rabbits but did not stress this finding.

It is now recognized that there is a coincidence between retinal capillary ectasia and glomerular lesions in diabetes.[11] There is a

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growing belief that these two lesions are essentially similar in origin. This belief is fortified by the fact that localized dilatations of isolated glomerular capillary loops sometimes are found in association with the solid nodular lesions.[2] Dilated capillaries also have been noted in the glomeruli of cortisone-treated rabbits.[1-5] It is well to point out, however, that even if it can be proved that the nodular glomerular lesions of diabetic glomerulosclerosis originate as simple capillary ectasia, there is no satisfactory explanation as to why these should occur or how the transformation to the solid nodule is accomplished. There is, in fact, no obvious explanation for the development of capillary ectasia in the retinas of diabetic patients. It also should be pointed out that Ballantyne,[12] who was among the first to recognize the nature of the diabetic retinal lesion, noted the presence of lipid material in the walls of the involved capillaries.

Lipid deposits and occasionally foam cells have been found in the glomeruli of cholesterol-fed rabbits, but lesions similar to those associated with cortisone administration have not been described. Distinctive glomerular lesions have not been reported in other forms of experimental hyperlipemia. It is obvious, therefore, that the development of nodular glomerular lesions does not depend primarily on increased concentration of lipid in the blood. If any relationship to blood lipids exists, it must be in connection with an altered pattern of their components. The present study was undertaken to determine if cortisone lipemia in rabbits differs in character from other forms of lipemia and whether or not such differences might be concerned in the development of the glomerular lesions.

TABLE I

Time Schedule and Plan of Experiment

Days of experiment	Group I		Group II		Group III	
	Cortisone	Cholesterol	Cortisone	Cholesterol	Cortisone	Cholesterol
1–14	None	None	None	1%	None	1%
15–28	5 mg.	None	5 mg.	1%	None	1%
29–42	None	None	None	None	None	None
43–56	10 mg.	None	10 mg.	1%	None	1%
57–70	None	None	None	None	None	None
71–84	15 mg.	None	15 mg.	1%	None	1%

METHODS

Young, adult rabbits, in three groups of 6 each, were made lipemic; the first group by courses of daily intramuscular injections of corti-

sone, the second by similar injections of cortisone and the addition of 1 per cent cholesterol to the food, and the third by the addition of 1 per cent cholesterol to the food without cortisone injections. The cholesterol was dissolved in ether and added to rabbit food pellets. The ether was then evaporated in warm air for at least several days before use. The rabbits were allowed up to 200 gm. of food daily. The time schedule of the experiment was as shown in Table I.

Cortisone (cortisone acetate, Merck) was given in three courses with the hope that glomerular lesions in three stages of development might be observed. One rabbit (no. 12 in Table II) died at the end of the

TABLE II

The Relation of Glomerular Lesions to Terminal Blood Lipid Concentration

Treatment	Rabbit no.	No. of glomerular lesions	Total lipid	Neutral fat	Total cholesterol	Phospholipid	Blood sugar
			mg. %	mg. %	mg. %	mg. %	mg. %
Cortisone only	1	155	3380	2927	130	358	321
(Group I)	2	56	2760	2099	216	463	364
	3	16	2290	1674	151	393	472
	4	15	1650	1458	65	143	386
	5	3	563	252	139	170	278
	6*	2	1220	1006	73	125	131
Cortisone & cholesterol	7*	57	10900	3079	4272	1550	114
(Group II)	8	52	8680	2683	3624	1330	436
	9	36	6520	2659	2238	980	243
	10	3	8740	3363	3120	1305	349
	11	3	19200	13877	3156	1920	157
	12†	0	1480	557	400	208	120
Cholesterol only	13*	1	5920	1138	3042	865	121
(Group III)	14	0	4340	1315	1812	660	100
	15	0	4160	918	2004	523	107
	16	0	2890	897	1188	470	113
	17	0	2800	944	1128	443	107
	18	0	1970	726	966	248	121

* Sacrificed on 56th day of experiment.

† Died on 31st day of experiment.

first 2-week course of daily 5 mg. cortisone injections, the 31st day of the experiment. One rabbit in each of the three groups (nos. 6, 7, and 13 in Table II) was sacrificed at the end of the second course of cortisone, the 56th day of the experiment. The remainder were sacrificed on the 84th day.

In a subsequent experiment 5 adult rabbits, weighing from 2000 to

2500 gm., were given 10 units of ACTH (ACTHAR Gel, Armour) intramuscularly daily for 14 days, at which time one was sacrificed. The 4 survivors were continued on 20 units of ACTH in two daily injections for 7 more days and 2 of these were then sacrificed. The remaining 2 rabbits received 40 units of ACTH daily for an additional 7 days and were sacrificed.

From each rabbit 8 to 10 cc. of blood was drawn from an ear vein at weekly intervals and analyzed for total and free cholesterol (Schoenheimer and Sperry), phospholipid (Fiske and Subarrow), proteins (biuret), and sugar (Folin and Wu). Total lipids were determined gravimetrically from the petroleum ether soluble fraction of a Bloor extract. Neutral fat was calculated by subtracting the combined weight of cholesterol ester and phospholipid from the weight of total lipid. No correction was made for the small amount of extraneous non-lipid material included in the total lipid determination. Blood pressure determinations by Grant and Rothschild's ear capsule methods,[18] and body weights were recorded at intervals of 2 or 3 days.

Two complete transverse sections from the mid-portion of one kidney and one complete longitudinal section from the opposite kidney were fixed in Zenker's fluid. From 12 to 15 paraffin-embedded sections, 5 μ thick, were stained with hematoxylin and eosin, Wilder's reticulum stain, Lieb's crystal violet stain for amyloid, periodic acid-Schiff's reagent (PAS), Goldner's modification of Masson's trichrome stain, hemalum-saffron-phloxine, and with toluidine blue for metachromatic substances. The transverse sections contained from 700 to 900 glomeruli; the longitudinal ones from 1100 to 1400 glomeruli. It was estimated that about 12,000 glomeruli from each rabbit were scanned. Frozen sections of formalin-fixed tissue were prepared from each kidney and stained with oil red O and Sudan IV.

Results

Renal Lesions in Rabbits Receiving Only Cortisone

The initial and constant alteration was moderate and fairly uniform dilatation of almost all glomerular capillaries. In a variable number of glomeruli one or two capillary loops were disproportionately enlarged. Such isolated loops measured up to 60 μ in diameter and often were several times as large as the rest of the capillaries in the same tuft. These ectatic capillaries usually were situated at the periphery of the tuft and often at the side opposite the hilar attachment. They were found frequently in the outermost layer of glomeruli, some 200 to 300 μ beneath the cortical surface.

The erythrocytes in the moderately dilated capillaries appeared in-

tact but those in the widely dilated ones often were irregular in shape and partly fused together. This process of fusion of erythrocytes was a progressive one so that eventually the impacted mass resembled a thrombus. When stained with hematoxylin and eosin it had a smudged bluish red color. In all other stains used, this material had the same color as intact erythrocytes. It did not stain deeply in PAS preparations. When stained with oil red O or Sudan IV the dilated loops at the periphery of the tufts were filled with sudanophilic material, whereas the rest of the tuft usually contained none. It was apparent, therefore, that the occluding masses of material in the dilated capillaries consisted of mixtures of fat and fused blood elements, probably derived chiefly from erythrocytes.

In a relatively few tufts these discretely dilated and apparently occluded capillaries showed an additional change. A thin, crescentic layer of homogeneous, wax-like material was deposited at the outer edges of the mass within the capillary and lining the basement membrane. The manner in which this deposit was first laid down and later elaborated could be followed by studying many lesions. The thin crescent became thicker and blunter, forming a heavy arc whose outer contour was molded by the basement membrane. It was usually on the side of the capillary that faced Bowman's space. As it enlarged it became reniform, ovoid, and eventually spherical. The contents of the capillary were largely replaced by the expanding mass of wax-like material. Eventually the intravascular position of these discrete deposits was no longer apparent. Often they appeared to protrude from the tuft into Bowman's space and, in some instances, had broken free from the tuft. Similar wax-like masses occasionally were found in various segments of the tubular system. There were also many scattered tubules that contained intact erythrocytes or hemoglobin casts.

With hematoxylin and eosin stains this material was homogeneous and pale bluish gray. With PAS stain it was fuchsinophilic and stained with the same intensity as the basement membranes. With Wilder's stain it was sometimes partly impregnated with precipitated silver but did not stain as intensely as reticulum. With Sudan dyes it was often free of fat but sometimes was diffusely sudanophilic or contained fat droplets. With all other procedures used, this material resembled erythrocytes in its staining reactions. It did not stain like collagen. Feulgen reactions were negative.

Although the intravascular position of the spherical masses was often not apparent, there was little evidence that the masses tended to be replaced by reticulum or fibrous tissue and thus incorporated into the framework of the tuft as are the characteristic nodular lesions

of Kimmelstiel-Wilson's disease. Nevertheless, they resembled these lesions in their shape, size, and position in the tuft even though they remained as discrete foreign deposits. In some respects they could be likened to casts within capillaries rather than within tubules.

Another rare but distinctive change, not mentioned in previous reports, was found in twelve glomeruli of 3 of the 6 rabbits in this group. This lesion consisted of the formation of clusters of large vacuoles with thin membranes within the substance of the tuft and sometimes replacing a large part of it. These were indistinguishable from adipose tissue cells. This particular change seemed to bear no relation to the discrete nodular lesions described. The loops of Henle of all cortisone-treated rabbits contained many fine fuchsinophilic granules in PAS preparations. Calcified masses and calcified epithelial cells were found in scattered convoluted tubules of 3 rabbits.

Renal Lesions in Rabbits Fed Cholesterol but Not Injected with Cortisone

The glomeruli of the 6 rabbits fed cholesterol but not injected with cortisone were small, compact, and relatively bloodless. Their capillaries were partly collapsed and not easily discernible. They occasionally contained one or two, rarely groups, of fat-laden phagocytes in their substance. The rest of the renal tissue was normal, except that focal accumulations of lipid often were found in the interstitium of the medulla and occasionally in the walls of arteries. With oil red O and Sudan stains the glomeruli were heavily stippled with very fine granules of sudanophilic material measuring up to 5 μ. These showed little tendency to coalesce. They were embedded in the framework of the tuft rather than in the lumina of capillaries. They appeared to be lodged in endothelial cells and to impregnate basement membranes. The basement membranes of the tubules were similarly impregnated with these granules. Almost no fat was found in tubular epithelium. At the hilum of many tufts in the region of the entering afferent arteriole triangular wedges of sudanophilic material measuring up to 50 μ in diameter were found just external to the tuft.

A single nodular lesion (Fig. 24) was found in one glomerulus of one rabbit in this group (no. 13, Table II). This was indistinguishable from the lesions in the cortisone-treated rabbits. Because of this finding, the kidney sections of 23 rabbits used in other experiments were reviewed. Eleven of these had been fed 1 gm. of cholesterol daily for more than 8 weeks. No comparable glomerular lesions were found although dilated peripheral capillaries were noted in some instances.

Renal Lesions in Rabbits Fed Cholesterol and Injected with Cortisone

The glomerular lesions in rabbits fed cholesterol and injected with cortisone were essentially similar in type and number to those found in rabbits receiving only cortisone. The wax-like accumulations, however, were more irregular and were sometimes fused to Bowman's capsule by proliferating epithelial cells. Foam cells occasionally were associated with the wax-like masses. The glomerular capillaries were more widely dilated than in the rabbits on cortisone only. The dilatation usually was uniform throughout the tuft and only infrequently limited to one or two loops. As a consequence, the glomeruli were larger in diameter than in the other groups of rabbits. The dilated capillaries often appeared to be empty or only partly filled with intact erythrocytes. Fused masses of erythrocytes were no more frequently found than in the rabbits on cortisone only. Only two glomeruli with large vacuoles simulating adipose tissue cells were found. With Sudan stains, the dilated capillaries were filled with fat. The interstitium of the tuft was heavily laden with fine granules of sudanophilic material. As a result, the glomeruli stained very intensely and were sharply outlined from the adjacent lipid-free tubular structures.

The Effect of Cortisone on the Chemical Composition of Serum of Normal and Cholesterol-Fed Rabbits

The mean blood lipid levels of the two terminal analyses are shown in Table III. It may be seen that the relative proportions of cho-

TABLE III

The Effect of Cortisone on the Blood Serum Lipids of Normal and Cholesterol-Fed Rabbits

	Initial levels	Group I cortisone only		Group II cortisone and cholesterol		Group III cholesterol only	
	Mean	Mean*	Ratio final/ initial level	Mean*	Ratio final/ initial level	Mean*	Ratio final/ initial level
	mg. %	mg. %		mg. %		mg. %	
Cholesterol (total)	77	156	2.0	2809	36.5	1420	18.4
Phospholipid	121	355	2.9	1273	10.3	469	3.9
Neutral fat (approximate)	416	2171	5.2	4580	11.0	932	2.2

* Computed from values obtained on blood serum samples on 77th and 84th days of experiment.

lesterol, phospholipid, and neutral fat differed strikingly in the three groups. By far the largest fraction of lipid in the rabbits on cortisone

only was neutral fat. The largest lipid component of the cholesterol-fed rabbits was cholesterol.

The group that received both cortisone and cholesterol had the more severe degrees of hyperlipemia, but all three lipid fractions were greatly increased. The absolute amount of neutral fat was larger in this group than in the rabbits that received only cortisone, but the relative increase of neutral fat compared to that of cholesterol was much smaller. On the other hand, the relative increase of neutral fat was much greater in the cholesterol-fed rabbits that were injected with cortisone than in those that received only cholesterol. Thus the "cortisone-cholesterol" group was intermediate to the other two groups in the relative proportions of the three major lipid components of serum. Nevertheless, neutral fat was the largest of the three fractions in both groups that received cortisone.

The mean percentage of free cholesterol in the serum of the rabbits receiving only cortisone increased from 28 to 58 per cent, but did not change significantly in the other two groups. There were no significant changes in the serum protein levels of any of the rabbits. The mean non-fasting blood sugar of the 6 cholesterol-fed rabbits did not vary appreciably throughout the experiment and was 112 mg. per cent at the time of sacrifice. The mean terminal blood sugar of the cortisone-injected group was 370 mg. per cent; in the group receiving both cholesterol and cortisone it was 305 mg. per cent. Equal degrees of glycosuria were observed in both groups of cortisone-injected animals. The blood pressures of all 18 rabbits remained within normal limits throughout the experiment.

Correlation Between Glomerular Lesions and Hyperlipemia or Hyperglycemia

Approximately equal numbers of glomeruli in the kidneys of each rabbit were examined. The vast majority had no nodular lesions. The levels of blood lipid and sugar at sacrifice and the number of lesions observed are recorded in Table II. There is no obvious correlation between the number of lesions and severity of hyperlipemia or hyperglycemia. Cortisone administration to cholesterol-fed animals did not increase the incidence of glomerular nodules.

In the group that received only cortisone, the 3 rabbits with the highest total lipid levels had a combined total of 227 lesions; the 3 with the lowest total serum lipid levels had only 20. This suggests that within this group there may be a positive correlation between lipemia and glomerular damage. However, variations in the level of lipemia may

reflect merely the effectiveness of cortisone responses in general in different rabbits.

Two rabbits that received both cortisone and cholesterol had very high terminal concentrations of serum neutral fat but only three glomerular lesions each. The development of glomerular lesions, therefore, does not depend upon the absolute concentration of serum neutral fat. However, in the "cortisone-cholesterol" group, both serum cholesterol and phospholipid also were greatly augmented. The injury to glomeruli may depend upon elevation in serum neutral fat without associated increases of other lipid fractions.

The one rabbit on cholesterol only, that had a single lesion, differed from the other animals in this group in that it developed an unusual degree of hyperlipemia at an extraordinarily rapid rate; when sacrificed at 8 weeks its lipid levels were higher than those of the other rabbits after 12 weeks of cholesterol feeding. The finding of this single lesion strongly suggests that cortisone administration is not absolutely essential for the development of glomerular changes of this type.

The Effect of ACTH on the Kidneys and Blood Lipids of Rabbits

The administration of increasing doses of ACTH up to 40 units daily in two divided doses over a 4-week period failed to produce any significant elevation in the blood lipids of 5 rabbits. There was, in fact, a very slight but consistent fall in phospholipid, cholesterol, and total lipid. There was also no significant rise of blood sugar. Glomerular lesions were not found in any of these animals.

Discussion

The data presented confirm some of the observations in the literature. Cortisone administration to rabbits caused a sustained neutral fat lipemia, hyperglycemia, widespread glomerular capillary dilation, and focal nodular lesions in a few scattered glomeruli. ACTH administration had none of these effects. Cholesterol feeding, with a single exception, did not cause glomerular lesions and did not significantly alter the incidence of such lesions when combined with cortisone injections.

No conclusive answer can be given to the question as to whether cortisone has a direct injurious effect on glomerular capillaries or whether the glomerular changes are wholly or in part secondary to the changed pattern of blood lipids found in these rabbits. In addition to the indirect evidence cited in the introduction, certain of our observations indicate also a relationship between sustained neutral fat lipemia

and glomerular lesions; namely, the obstructing masses found within dilated peripheral glomerular capillaries regularly contain sudanophilic material, the wax-like casts formed in these areas sometimes contain fatty material, and nests of adipose tissue are found, even though rarely, within the tufts.* Pinkerton[15] noted that adipose tissue sometimes formed around bronchi of rabbits into which rabbit neutral fat had been instilled. This did not occur when other fats were introduced in this fashion. The formation of adipose tissue within glomeruli possibly represents a comparable process and suggests that the neutral fat of cortisone lipemia is derived from the animal's own body fat. The latter was markedly depleted at necropsy.

A mechanism by which neutral fat lipemia could initiate this glomerular process is suggested by the observations of Cullen and Swank[16] on the effects of alimentary lipemia on the capillary circulation in the cheek pouch of hamsters. These authors noted that late in the absorptive period following a fatty meal there was stasis of capillary circulation and the capillaries were filled with aggregates of clumped erythrocytes. Circulation was restored to normal in the post-absorptive period. Cortisone produces, in effect, a sustained lipemia of alimentary type in the rabbit and may, therefore, produce similar alterations in capillary circulation in this species. That rabbit sera rich in neutral fat can damage erythrocytes is suggested by the fact that agglutinated clumps of red blood cells form rapidly in suspensions of blood from cortisone-treated rabbits. Such clumping is not found in similar preparations from normal rabbits. Swank[17] noted similar erythrocyte aggregates in blood following ingestion of fat by man and the dog.

Glomeruli may be more vulnerable to capillary stasis in sustained neutral fat lipemia than other structures for two reasons. Glomerular filtration should increase the concentration of non-filterable serum lipid by about 17 per cent during the passage of blood through the capillary loops[18] and, secondly, the arrangement of the capillaries in each glomerulus makes it possible for stasis to occur in a single loop while the blood continues to circulate through others. In cortisone-treated rabbits, single dilated capillary loops often are found in the outermost layer of cortical glomeruli at the greatest distance from the hilar arteries. The most frequent location of this dilated capillary in the glomerular tuft is peripheral at a distance from the entering arteriole. These observations suggest that glomerular capillaries so

* In a recent publication,[14] it has been shown that heparin prevents both gross lipemia and glomerular lesions in cortisone-treated rabbits. This may be further evidence that the renal change is related to the lipid patterns of the blood.

situated are in the least advantageous position in regard to arterial blood flow and are apt to become dilated and eventually obstructed if the blood flow is impaired by increased concentration of neutral fat in the serum.

Assuming that glomerular capillary dilation is brought about by stasis resulting from neutral fat lipemia, it is possible to speculate about the further progression of the lesions. The dilated capillaries are first filled with stationary masses of erythrocytes and lipid-rich plasma. The erythrocytes clump together and fuse, forming obstructing masses infiltrated with fat. The dilated and obstructed portion of the loop seems to lose continuity with the remainder. The obstructing mass of fused erythrocytes and fat is transformed into a homogeneous wax-like material. Such alteration of the original substance into the wax-like material would suggest that a chemical transformation has occurred and that the wax-like material does not result from the deposition of an entirely unrelated material.

A relationship between the glomerular lesions in rabbits on cortisone and the nodular glomerular lesions of human intercapillary glomerulosclerosis has not been fully established. The size, shape, location, and focal character of the two lesions are very similar, as is their association with diabetes. However, transformation of the wax-like deposit in the rabbit's glomerulus to a hyalinized, spherical mass with all the staining reactions of the human glomerular nodule has not been observed. Bloodworth and Hamwi[5] noted deposits similar to those of the rabbit in the more fulminating cases of Kimmelstiel-Wilson's disease with multiple glomerular changes. Perhaps some additional factor, such as hypertension, is required to allow progression of the rabbit lesion to the stage commonly seen in human intercapillary glomerulosclerosis.

There is evidence[19,20] that some diabetic subjects respond to fat ingestion with unusually pronounced and prolonged degrees of alimentary hyperlipemia. Peters and Van Slyke[21] found that 19 of 79 diabetic patients had hyperlipemia irrespective of diet or the severity of the disease. Diabetic patients with retinopathy have higher fasting levels of total plasma lipid than those without retinopathy, according to Iannaccone and Kornerup.[22] Some patients with diabetes may, therefore, have neutral fat lipemia, although this may not be as pronounced or as sustained as in cortisone-treated rabbits. According to Goldring and Chasis,[23] the glomerular filtration fraction is sometimes increased in hypertension, probably due to spasm of the efferent arteriole. This might be an additional factor in certain hypertensive

diabetic patients which would tend to concentrate the serum lipids within glomerular capillaries.

Summary

Nodular and fatty glomerular lesions in cortisone-injected rabbits are described and their pathogenesis discussed. Cortisone administration leads to a sequence of changes, peculiar to the rabbit, that appear to be interrelated and of significance in the development of these lesions. These include: (1) increases in neutral fat content of the blood; (2) irregular dilation of glomerular capillaries with the peripheral portions of the loops most markedly involved; (3) occlusion of dilated capillaries with fused masses of erythrocytes and lipid-rich plasma; (4) conversion of the occluding intraluminal mass into a wax-like material or, more rarely; (5) deposition of large fat droplets that eventually come to resemble adipose tissue. It is suggested that the mechanism by which the altered proportions of the blood lipids may participate in this process is by causing protracted periods of glomerular capillary stasis similar to those that have been observed transiently in other capillary beds in alimentary lipemia.

We wish to thank Miss Gizella Jakab who performed the chemical determinations.

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[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. Moderate, fairly uniform dilation of all glomerular capillaries which are partly filled with pale-staining erythrocytes in a rabbit that received both cortisone and cholesterol. This change is found in the majority of glomeruli of cortisone-injected animals but is non-specific. Periodic acid-Schiff's reagent (PAS) stain. × 375.

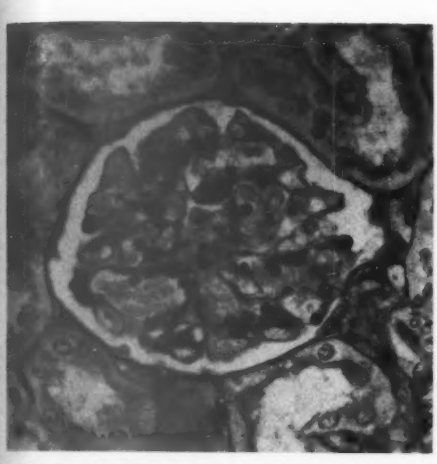
FIG. 2. Irregular telangiectatic capillary occupies almost the entire left half of the glomerulus. It is filled with a mass of erythrocytes with indistinct outlines. Rabbit on cortisone only. PAS stain. × 452.

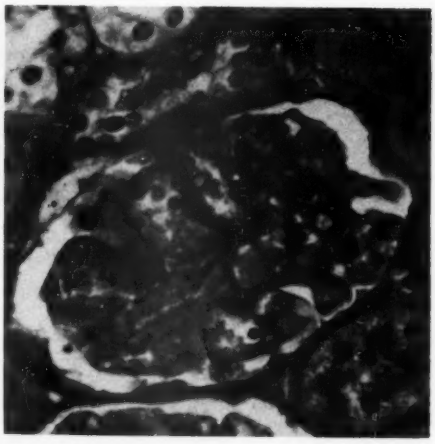
FIG. 3. Two disproportionately enlarged capillary loops in an otherwise normal glomerulus. The smaller one at the upper margin is filled with a mass of homogeneous pale-staining material. The larger one at the right contains, in addition, a small amount of fuchsinophilic material, apparently forming within the occluding mass. Rabbit on both cortisone and cholesterol. PAS stain. × 420.

FIG. 4. Large, clear space in glomerulus is either a dilated vessel or a large fat globule. Lining up of cells at its margins suggests it is a vascular channel. Rabbit on cortisone only. Toluidine blue stain. × 382.

FIG. 5. Dilated capillary at right margin of tuft filled with pale-staining coagulum. It is pressing against a large fuchsinophilic mass in Bowman's space. The latter has been molded into a thin cord with knob-like extremities. Rabbit on cortisone only. PAS stain. × 546.

FIG. 6. Oil red O without counterstain on a frozen section. The dilated capillaries at the periphery of the glomerulus are filled with sudanophilic material. This change is found in many glomeruli of cortisone-treated rabbits whether or not they have received cholesterol. × 294.





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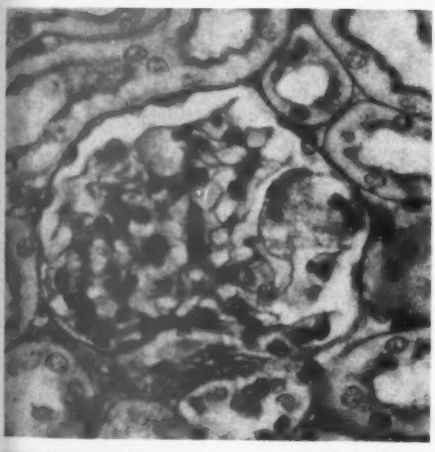

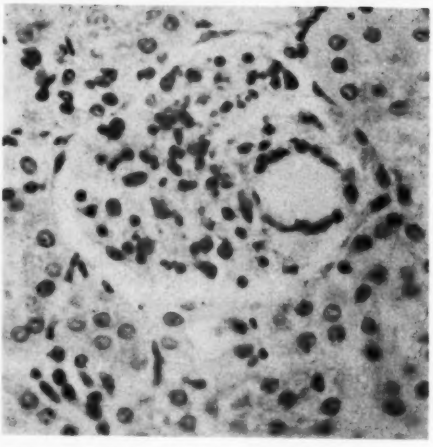

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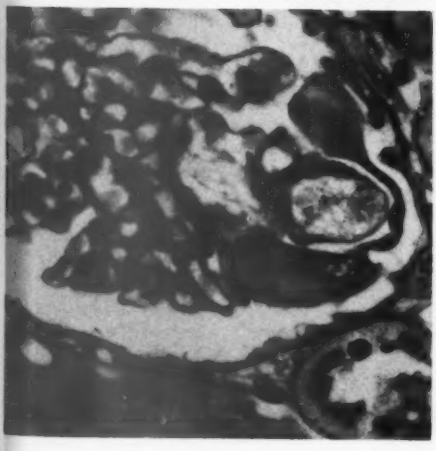

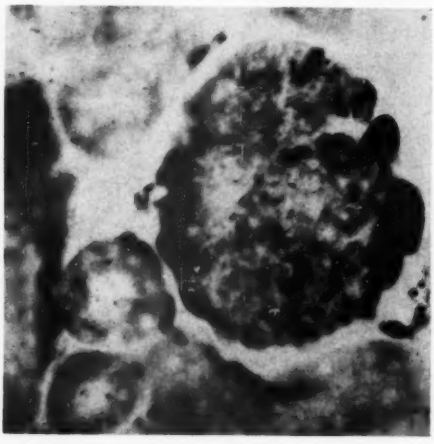

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FIG. 7. Crescentic mass of pale-staining, wax-like material at the outer margin of glomerulus. Although the outlines of the capillaries are not discernible in the photograph of this preparation stained with hematoxylin and eosin, the deposit is intraluminal. Rabbit on cortisone only. × 465.

FIG. 8. Similar lesion in PAS-stained section. The crescentic mass lines one segment of a dilated capillary and is strongly fuchsinophilic. × 449.

FIG. 9. Nodular lesion composed of wax-like material in dilated capillary loop. The vessel is almost completely occluded but several leukocytes and the remains of the lumen are barely visible at the upper left of the mass. Hematoxylin and eosin stain. × 471.

FIG. 10. A dilated glomerular capillary is almost completely filled with an ovoid mass of fuchsinophilic material. PAS stain. × 441.

FIG. 11. A rounded hyaline mass is present at the right lower border of the tuft and is fused to Bowman's capsule. Its intravascular origin is no longer apparent. Hematoxylin and eosin stain. × 392.

FIG. 12. In this Wilder's reticulum preparation a nodular lesion is heavily impregnated. The unstained foamy area at the right of the nodule probably contains fat. Rabbit on cortisone only. × 33.

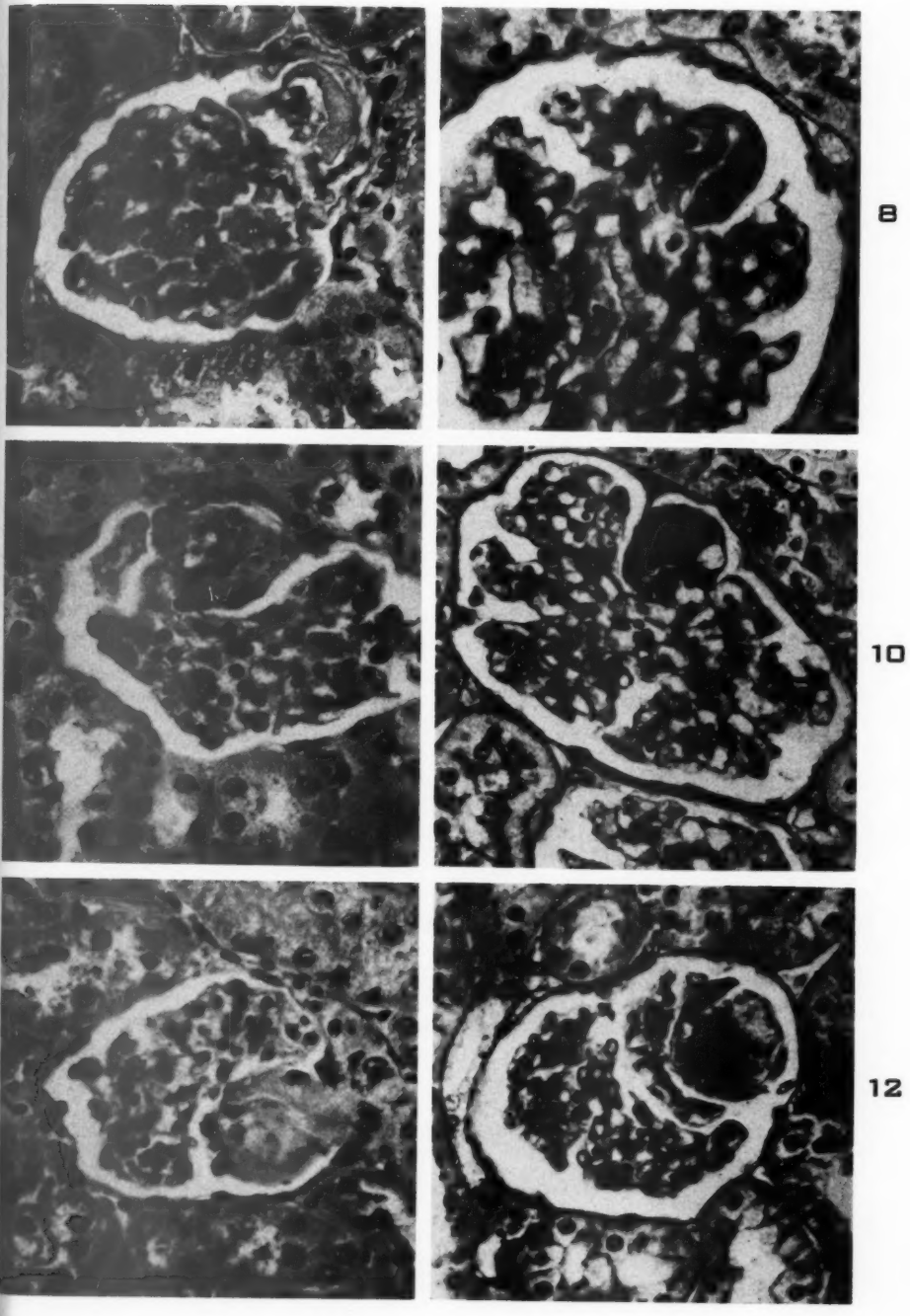
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FIG. 13. The spherical nodule in this glomerulus stains very deeply with phloxine as do the erythrocytes in the capillaries. Hemalum-phloxine-saffron stain. × 439.

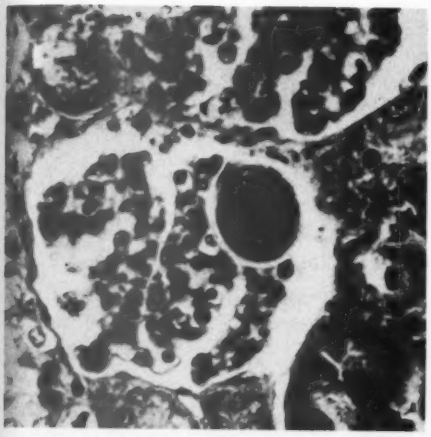
FIG. 14. A large ovoid nodule has become partly detached from the tuft. Between it and Bowman's capsular membrane are swollen epithelial cells. The nodule is predominantly fuchsinophilic but contains rarefied areas from which fat probably has been dissolved. Rabbit on cortisone only. PAS stain. × 504.

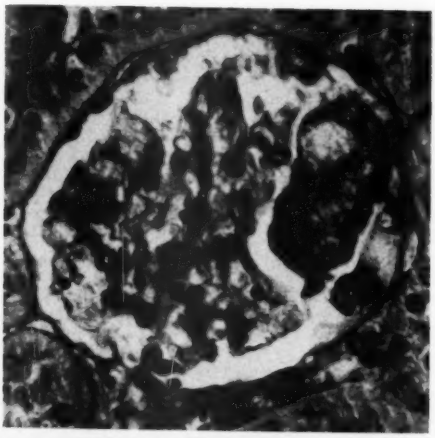
FIG. 15. A nodular mass of waxy material within the tuft has been molded into an irregular shape by external pressure of adjacent capillaries. Such remolded masses sometimes contain fracture lines indicating that they are moderately brittle. PAS stain. × 565.

FIG. 16. The glomerular space contains a large, rounded mass honeycombed with fat vacuoles. This is attached to and compresses the remains of the glomerular tuft. Rabbit on cortisone only. Hematoxylin and eosin stain. × 388.

FIG. 17. Two discrete fat vacuoles separated by an indistinct partition are embedded in a glomerular tuft close to its hilar attachment. Rabbit on cortisone only. Hematoxylin and eosin stain. × 463.

FIG. 18. Two large fat vacuoles with thin membranes resemble adipose tissue cells, and are lodged in the glomerular tuft. Rabbit on cortisone only. Hematoxylin and eosin stain. × 329.





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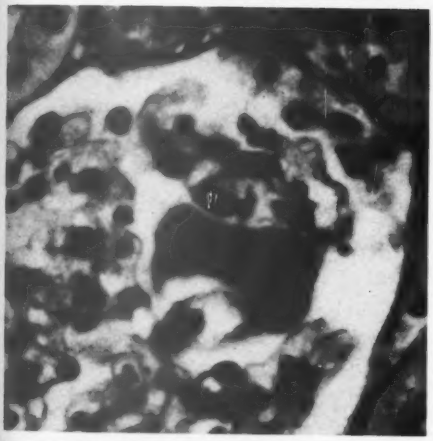

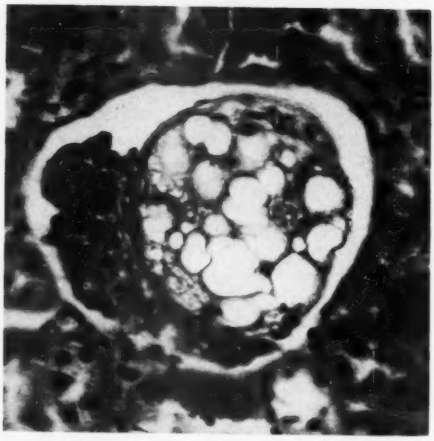

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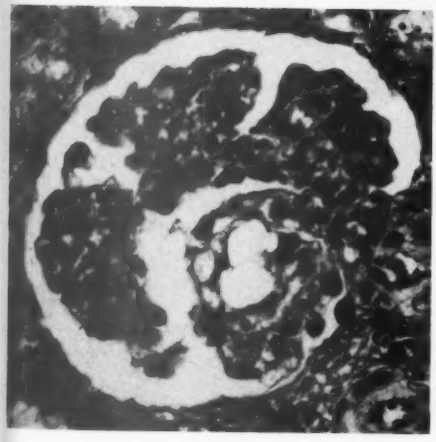

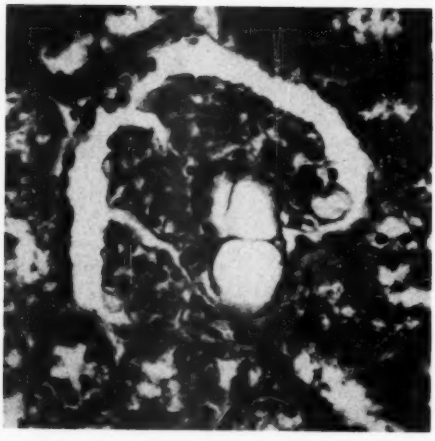

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FIG. 19. A cluster of three adipose tissue cells is located in the center of a glomerular tuft. Rabbit on cortisone only. Hematoxylin and eosin stain. × 369.

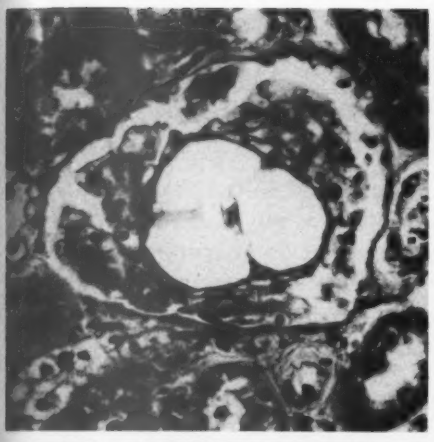
FIG. 20. A very large fat globule almost fills the glomerular space and compresses the rest of the tuft. Its outer membrane has retracted from the tuft but subdivision into small adipose tissue cells is not apparent. Rabbit on cortisone only. PAS stain. × 514.

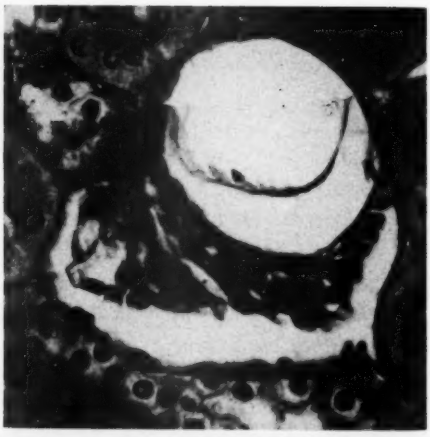
FIG. 21. A large cluster of adipose tissue cells protrudes from and compresses the glomerular tuft. The outlines of the individual adipose cells are barely visible. Rabbit on cortisone only. Hematoxylin and eosin stain. × 711.

FIG. 22. The black areas represent large globules of fat in adipose tissue cells within a glomerulus. Rabbit on cortisone only. Oil red O without counterstain. × 232.

FIG. 23. Glomerulus heavily infiltrated with fat so that its architecture is all but obliterated. Rabbit on cortisone and cholesterol. Sudan IV without counterstain. × 215.

FIG. 24. Nodular glomerular lesion in rabbit on cholesterol but without cortisone. Hemalum-phloxine-saffron stain. × 370.





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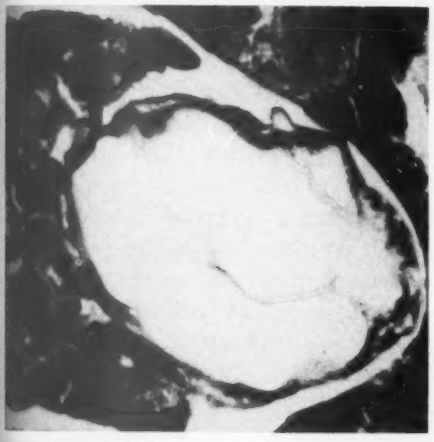

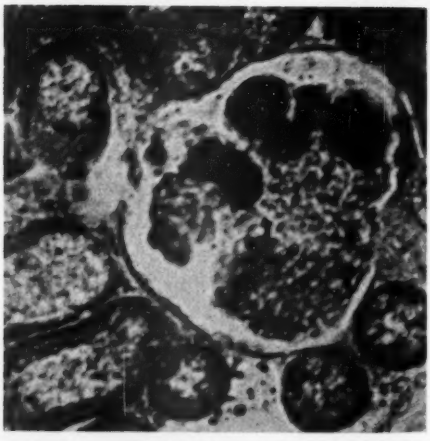

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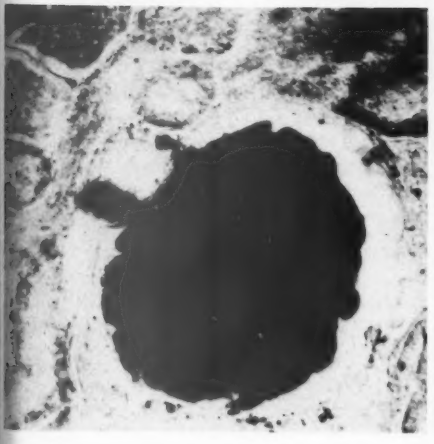

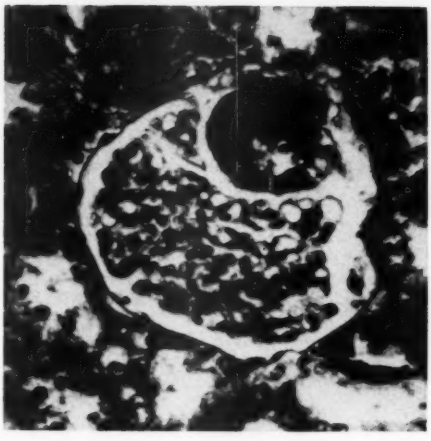

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LESIONS OF THE CORPUS CALLOSUM FOLLOWING BLUNT MECHANICAL TRAUMA TO THE HEAD*

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Traumatic lesions in the corpus callosum occur in a significant portion of all blunt injuries of the head (Rowbotham[1]) and are important for reconstruction of the nature and direction of the impact. Despite this, little attention has been paid to these lesions, mention being made of them only incidentally among other findings. A few authors[2-7] have described them as the most striking alterations in individual cases.

During the last 2 years we have paid special attention to the involvement of the corpus callosum in cases of blunt mechanical trauma to the head studied in the Office of the Chief Medical Examiner of the State of Maryland. Gross alterations were present in 51 cases which constituted 16 per cent of those with blunt injury to the head. This confirms the incidence of such lesions reported by Rowbotham.[1] Table I presents the essential data on direction of impact and survival in our cases. In 9 instances the lesions covered the entire length of the corpus callosum from knee to splenium, always sparing the rostrum and the ventral portion of the knee. Twenty-one cases showed involvement of from one to two thirds of the corpus callosum. The third group of 21 cases demonstrated varieties of small, occasionally multiple, individual foci occurring predominantly in the caudal half of the corpus callosum. All age groups were represented without any one group prevailing. In 16 cases, approximately one third of the total, the lesions occurred without skull fracture. There was no relationship between absence or occurrence of skull fracture and the severity of damage to the corpus callosum. The impact direction as listed in Table I was deduced from clinical data and necropsy findings. Survival time varied from a few minutes to several months with the longest being 8

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TABLE I

Traumatic Lesions in Corpus Callosum

Case no.	Longitudinal extent of lesion	Age	Type of accident	Skull fracture	Impact direction	Location of lesion	Lesions in adjacent structures	Survival time
1	Entire corpus callosum	30	Pedestrian hit by car	+	Lower occipital to centro-frontal			6½ hours
2		29	Pedestrian hit by car	+	Right vertex-parietal to base of skull		+	3 days
3		24	Aircraft accident		Vertex to base of skull		+	3 days
4		22	Driver of auto in collision	+	Vertex-frontal to base of skull		+	3 days
5		20	Passenger of car in collision	+	Right fronto-central to base of skull, oral		+	1½ hours
6		66	Fall from stairway	+	Right fronto-parietal to left base of skull		+	3 days
7		19	Motorcyclist in collision	+	Left parieto-temporal to right temporal			1 day
8		22	Pedestrian hit by car		Parieto-occipital to base of skull		+	10 hours
9		57	Fall down stairs in altercation	+	Vertex-occipital to base of skull, frontal			7 hours
10	One third to two thirds of corpus callosum	29	Pedestrian hit by car		Right occipito-parietal to right frontal, parallel to midline	Oral and middle thirds	+	5 days
11		18	Passenger of car in collision	+	Right frontal to left temporal	Oral half	+	1½ days
12		26	Pedestrian hit by car	+	Right frontal to left occipital	Oral half		3 days
13		48	Fell or was struck (drunk)		Right centro-parietal to base of skull	Oral half	+	11 hours
14		38	Crushed by crane	+	Left occipito-parietal to right temporal	Oral half		1 day
15		39	Struck head on curb	+	Left temporo-parietal to right frontal	Oral half		5 days
16		40	Passenger of car in collision	+	Left parieto-occipital to orbital	Oral third		1½ hours
17		17	Driver of auto in collision		Unknown	Middle third		22 hours
18		19	Fall from 20 ft. ladder	+	Right lower parietal to oral half of base of skull	Middle third	+	10 hours
19		59	Fall from steps	+	Right parieto-temporal to left temporo-parietal	Middle third	+	1 hour
20		8	Pedestrian hit by car	+	Right parieto-temporal to base of skull	Middle third	+	1 day
21		28	Aircraft accident		Right fronto-central to left temporal	Middle third	+	1 day
22		33	Pedestrian hit by car	+	Right occipito-parietal to left frontal	Middle and caudal thirds	+	16 hours
23		22	Passenger of car in collision	+	Left temporo-occipital to right fronto-temporal	Middle and caudal thirds		42 days
24		50	Pedestrian hit by trolley	+	Right parieto-temporal to base of skull	Caudal half		

24		50	Pedestrian hit by trolley	+	Right parieto-temporal to base of skull	Caudal half	+	2 days
25		40	Pedestrian hit by car		Unknown	Caudal half		13 hours
26		72	Driver of auto in collision		Right parieto-temporal to base of skull	Caudal third	+	8 days
27		21	Passenger of car in collision	+	Right parieto-temporal to base of skull	Caudal third	+	8 hours
28		40	Pedestrian hit by car	+	Occipito-parietal to frontal, bilaterally	Caudal third		33 hours
29		54	Struck in head in fight		Vertex-parietal to base of skull	Caudal third	+	14 days
30		49	Pedestrian hit by car	+	Left parieto-temporal to right base of skull	Caudal third	+	11 hours
31	Small lesions in corpus callosum	42	Pedestrian hit by car	+	Right parieto-temporal to left frontal	Oral third		2 days
32		53	Pedestrian hit by car	+	Right frontal to left temporal	Oral third	+	18 hours
33		61	Pedestrian hit by streetcar	+	Left parieto-temporal to right temporal	Oral third		1½ mos.
34		76	Pedestrian hit by car	+	Right parietal to frontal base of skull	Middle third		11 days
35		60	Pedestrian hit by car		Vertex to base of skull	Middle third	+	9 days
36		32	Fall from 40 ft. scaffold	+	Right occipito-parietal to left fronto-temporal	Middle third	+	3 hours
37		59	Driver of auto in collision	+	Left frontal to occipital	Middle third		6 days
38		6	Fall from moving truck		Frontal to occipital	Middle third	+	10 hours
39		43	Unknown		Left parietal to base of skull	Middle third	+	Unknown
40		7	Passenger of car in collision	+	Left parieto-temporal to right temporal	Middle third	+	8 hours
41		38	Passenger of car in collision	+	Unknown	Middle third	+	8 months
42		33	Driver of overturned car		Left parieto-temporal to right base of skull	Caudal third	+	16 days
43		5	Pedestrian hit by car		Right occipito-parietal to base of skull	Caudal third		2 hours
44		10 mos.	Fall from crib	+	Right parieto-occipital to base of skull	Caudal third		Unknown
45		9	Pedestrian hit by car	+	Lower occipital to frontal	Caudal third	+	10 hours
46		43	Pedestrian hit by car	+	Right fronto-parietal to base of skull	Caudal third		20 hours
47		18	Fall from 24 ft. ladder	+	Lower occipital to frontal, bilaterally	Caudal third		A few minutes
48		55	Pedestrian hit by car		Unknown	Caudal third	+	3 months
49		41	Pedestrian hit by car		Vertex to base of skull	Caudal third	+	14 days
50		57	Pedestrian hit by car	+	Right temporo-occipital to left fronto-temporal	Caudal third		18 days
51		43	Was struck and fell backwards	+	Occipital-parietal to frontal	Caudal third		10 weeks

months. Lesions of the corpus callosum were frequently accompanied by traumatic foci in other brain areas, e.g., the cingulate gyri, the septum pellucidum, fornices, the caudate nuclei, and the dorsal thalamus. Such lesions were observed in 30 cases and, when present, were significant in clarifying the pathogenesis of the lesions of the corpus callosum.

Pathologic Findings

Early Lesions. The early lesions are predominantly hemorrhagic in nature, with disruption of tissue. Two factors seem to contribute to this picture, disruption due to a primary stress effect occurring at the moment of impact and secondary tissue separation due to expanding hemorrhage. Depending upon the severity of the trauma, the hemorrhages may be small or large. The histologic findings are limited to edema, hemorrhage, and fiber interruption. Hemorrhagic lesions of this type (Fig. 1) may be seen in the corpus callosum as early as a few minutes after the accident.

Progressive Changes. In all cases surviving more than a few hours necrosis becomes a significant element in the pathologic picture. The hemorrhage may dominate (Fig. 2) and failure of resorption or organization may lead to coagulation necrosis of the core of the hemorrhage (Fig. 3) with peripheral rarefaction due to solution of necrotic nervous tissue by phagocytes. More frequently the end result is a yellowish scar which may be easily overlooked (Fig. 4). Usually necrosis and hemorrhage go side by side. In the very early phase this type of lesion appears as a pale, edematous area with minute foci of hemorrhage. As necrosis develops, the tissue may become suffused with blood pigment erroneously giving the impression of a large hemorrhage. The early microscopic picture is that of a few red blood cells centrally located in an area of pale-staining myelin. As the necrotic area enlarges, it may become confluent with neighboring foci, and myelin loss may become complete. Figures 5 and 6 depict the early phases while Figure 7 shows confluent necrosis in a case with long survival. Connective tissue, microglia, some astrocytes, and occasionally some oligodendroglia may survive. Therefore, instead of a cyst a loose scar develops in which the glial and vascular elements are more densely arranged than normal (Fig. 8). Other cellular elements present in these lesions are compound granular cells (gitter cells) of the fixed tissue type which often are lined up in groups of three or four along persisting strands of tissue fibers (Fig. 9). Prussian blue stains reveal iron which, in contrast to the findings in massive hemorrhage, is diffusely distributed throughout the lesion. Conglomeration of iron-

containing macrophages occurs only around vessels (Fig. 10). This is the picture of an organized diapedetic hemorrhage with imbibition of blood pigment by the edematous and early necrotic tissue.

Anemic Necrosis. In a few cases the lesion of the corpus callosum constituted an area of anemic necrosis. In contrast to the hemorrhages, it is very difficult to recognize such necrosis when fresh, but it can be readily seen after survival for a few days. It is usually limited to a small area. Figure 11 (case 49) illustrates such a softening with survival for 14 days. The microscopic changes in lesions of this type are indicative of irregular loss of occasional fibers with an accompanying progressive cellular reaction. The vessels, arteries as well as veins, show activation of their connective tissue elements and are, therefore, more clearly visible than normally. White blood cells, especially polymorphonuclear cells and large mononuclear cells, may be seen in the perivascular spaces which are often slightly enlarged (Fig. 12). The microglia, which show enlarged nuclei and cell bodies, become oriented parallel to the fibers of the corpus callosum. The astrocytes may also participate in this reaction. These diffuse cellular reactions may be accompanied by a slowly progressing diffuse degeneration of myelin sheaths (Fig. 15). The participation of the vascular system and the diffuse widespread distribution of the degenerative process suggest that it is caused by traumatic irritation of insufficient severity to cause softening and hemorrhage. These diffuse alterations may be seen in distant areas accompanying massive or diapedetic hemorrhagic lesions, as well as independently.

All three types of lesions—massive hemorrhages, diapedetic hemorrhages with softening, and diffuse alterations of the nature described—are often found in the neighboring structures to the corpus callosum; namely, in the septum pellucidum, caudate nuclei, fornices (Fig. 13), dorsal thalami (Fig. 11), and cingulate gyri (Fig. 14). Hence, a description of the changes in these structures is unnecessary. It is worth-while to mention that in many cases a subarachnoid hemorrhage in the sulcus callosus is found. This can be identified easily even in older cases by the presence of iron pigment in phagocytes, caught in the subarachnoid network, and in glial cells of the outer zone of the first cortical layer of the cingulate gyri (Fig. 16).

Illustrative Cases

Acute Lesions

Case 5. This 20-year-old passenger in an automobile sustained head injuries when the car struck a tree. There were lacerations of the scalp

and face and a fracture of the right humerus. Hyperthermia was noted. The patient was unconscious and in a decerebrate state for the 1½ hours he survived. At necropsy, a few small fracture lines were found in the right orbital roof. There was no significant meningeal hemorrhage. Multiple small contusion hemorrhages were found in the cortex and white matter of the brain, some in areas which are rarely involved, such as the right insula and both parieto-occipital lobes. The lesion of the corpus callosum was confined to the right half of its entire length. At the level of the tuber cinereum it consisted of a single massive, wedge-shaped, sharply demarcated hemorrhage. Opposite this hemorrhage there was a second massive hemorrhage in the right caudate nucleus and hemorrhagic necrosis of the septum pellucidum. Both gyri cinguli revealed contusion hemorrhages in the cortex and white matter (Fig. 13). On further sections, there was severe damage to the fornices and the right dorsal thalamus.

Case 47. This is the case with shortest survival after injury. An 18-year-old sailor fell from an iron ladder striking the lower portion of his occiput. At necropsy, a comminuted fracture of the occipital bone around the foramen magnum was found. Cerebellum, medulla oblongata, and pons showed multiple, very small hemorrhages. A few small hemorrhages were located in the thalamus. In the corpus callosum there were several small lesions in the medial portion of the caudal third. At the level of the red nuclei the lesion consisted of circumscribed, slightly hemorrhagic discoloration, whereas in the other areas it was represented by individual, very small, petechial hemorrhages (Fig. 1).

Progressive Lesions

Case 1. This 30-year-old man was found in an unconscious condition after having been struck by an automobile. Upon arrival at the hospital, no abrasions or lacerations of the head could be found. Nevertheless, there was a fracture of the base of the skull. At operation, a severe swelling of the brain was found and, therefore, sectioning of the tentorium was attempted. The patient remained unconscious and expired 6½ hours after the accident. The necropsy confirmed the lack of superficial lesions about the head and revealed that the fracture extended around the foramen magnum and radiated into the occipital and temporal bones. It was depressed and had lacerated the lower half of the cerebellum. Bilateral hematomas of the buttocks indicated that the impact was transmitted through the vertebral column to the base of the skull.

The more obvious lesions in the corpus callosum were multiple small

hemorrhages surrounded by fresh, necrotic tissue. The lesions extended from the knee of the corpus callosum to its splenium (Fig. 17). The rostrum, the ventral portion of the knee, and most of the ventral and dorsal fibers were not involved. There was no actual tearing of the corpus callosum. There were no significant subdural or subarachnoidal hemorrhages over the cerebral hemispheres. In both frontal lobes several small, fresh, cortical contusions were found. The brain was intensely swollen and the ganglia of the upper brain stem compressed and shifted caudally resulting in marked secondary hemorrhages in the midbrain and upper pons.

Case 6. This 66-year-old laborer fell from a stairway and was comatose on admission to the hospital. He showed no localizing neurologic signs and died 3 days later without regaining consciousness. At necropsy, a subcutaneous hematoma over the right frontoparietal area and a small fracture of the right temporoparietal bone were found. In the corpus callosum were multiple, individual hemorrhages resembling those found with fat embolism (Fig. 18). These were found throughout the entire length of the corpus callosum in the ventral portion of its middle sector. In addition, there were destruction of the fornices and contusion hemorrhages in the left caudate nucleus and in the cortex of the right gyrus cinguli.

Anemic Necrosis

Case 26. A 72-year-old man was driving his car when it collided with another auto. He did not lose consciousness and was released from the hospital because of lack of cerebral symptoms. Four days later he was re-admitted with decerebrate rigidity and operated on for removal of a subdural hematoma over both cerebral hemispheres. After early improvement, he again developed decerebrate rigidity and died 8 days after the accident. At necropsy, no skull fracture could be found. There was some subdural hemorrhage. The immediate cause of death was acute bronchopneumonia. The lesion in the corpus callosum was an area of anemic necrosis located in the caudal third (Fig. 21) and accompanied by a small contusion focus in the right gyrus cinguli (not shown in Fig. 21). In addition, there was extensive contusion in the right third temporal convolution.

Discussion

Pathogenesis

There are three principal explanations as to the causative mechanism of the lesions of the corpus callosum. Rowbotham[1] implied that

the brain may be displaced at the moment of the impact in such a manner that the corpus callosum strikes against the edge of the falx. Junet[5] believed that the impact causes a sudden increase in pressure of the cerebrospinal fluid within the ventricles, leading to a rupture of the corpus callosum. Hämäläinen[3] explained the lesion in his case by a lateral displacement of the cerebral hemispheres due to an elastic deformation of the skull at the moment of impact whereby maximum stress is effective in the center line of the corpus callosum.

As obvious as Rowbotham's[1] theory seems to be, it has definite limitations for anatomical reasons. The falx is sufficiently close to the corpus callosum to allow damage by direct contact only in its caudalmost portion. Such damage would consequently be located in the center line and primarily involve the dorsal portion of the splenium. In one single case in our series there was a short, longitudinal, hemorrhagic defect in this location, suggesting a cut by the edge of the falx. In all other cases, Rowbotham's theory was not applicable.

As to Junet's[5] explanation, it is difficult to conceive how and why the corpus callosum, either in its entirety or in portions, could suffer by a sudden increase of the intraventricular pressure alone. Certainly, such an increase takes place at the moment of the impact if the skull is elastically deformed for a few milliseconds. Nevertheless, one should assume that the intraventricular fluid has, at least to some extent, a protective function by evenly dispersing a transmitted force within the ventricles according to the physical law of hydrodynamic pressure.

Hämäläinen's[3] theory, favored by Krauland,[6] offers the most reasonable explanation. It is obvious that the corpus callosum is exposed to laterally directed, stretching forces if the horizontal perimeter of the skull is bilaterally expanded. Considering the physical structure of the skull, the maximum deformation of this type should be expected in an impact inflicted at the vertex and directed toward the base of the skull. Indeed, several of our cases show this impact mechanism, among them case 3, the only one with complete tearing (Fig. 19). An impact against the lower occipital region and directed against the vertex may have the same effect as is exemplified by our cases 1 (Fig. 17), 45, and 47.

We believe, however, that not only these stretching forces but also pressure and shearing forces are responsible for the lesions described (Holbourn[8]). In any impact, there is a very short-lasting elastic depression of the skull, limited to the impact area. This deformation initiates a pressure wave and an abrupt shifting of the subjacent tissue in the path of the direction of the impact. Under certain circum-

stances the corpus callosum will participate in this shifting. In principle, the same shifting mechanism takes place in cases in which, for instance, an epidural or subdural hematoma exerts pressure on the dorsal portion of one hemisphere. Figure 20 demonstrates a case in which a hematoma was removed shortly before death, and we found this marked shifting of the involved hemisphere toward the opposite side. The severely herniated gyrus cinguli exerted pressure on the corpus callosum and its vessels, causing a softening of the contralateral half of the corpus callosum similar to a primary traumatic lesion. This displacement took several hours to develop whereas the deformation of the brain at the moment of an impact lasts only a fraction of a second. Nevertheless, we believe that at the peak of an impact against the right vertex the deformation is very similar. Thus Figure 20 may be taken as an approximate still picture of the intracranial situation at the moment of the maximal traumatic shifting. The concomitant presence of lesions in structures subjacent to the corpus callosum (septum pellucidum, fornices, caudate nuclei, and dorsal thalami) is also evidence of a shifting of the corpus callosum. These lesions must be considered as "internal contusion foci" due to an impact by the lower surface of the corpus callosum. The hemorrhages, so often seen in the white matter of the cingulate gyri and in their cortices facing the corpus callosum, are also caused by the shifting process. The vertical alignment of all these lesions presupposes that the impact area on the skull must have been located above the horizontal level of the corpus callosum and the impact directed toward the base of the skull. Indeed, this was true in 48 of our cases. In the cases in which the impact area was close to or within the midline, the lesions frequently extended over the entire cross section of the corpus callosum (Fig. 14). In cases with more laterally located impact areas, the area of damage to the corpus callosum was usually smaller and asymmetric (Fig. 13). Among the 3 cases in which not the calvarium but the lower occipital area was the site of the impact, one (case 45) showed, in addition to the lesion of the corpus callosum, a contusion of the dorsal thalamus. This suggests that in an inverse shifting mechanism the dorsal thalamus may strike the lower surface of the corpus callosum with sufficient intensity to cause damage.

In none of our cases was the impact directed against the lateral portions of the skull or against the occipital protuberance with spreading of the force in the horizontal plane.

In summary, it is our opinion that the lesions of the corpus callosum are the result of sudden stretching as well as pressure and shear-

ing forces occurring at the moment of the impact, the impact area usually being located above the level of the corpus callosum and occasionally in the lower occipital region. The extent and location of the lesion in each specific case depend on location and size of the impact areas, direction of the impact, and the proportional magnitude of the different forces. The variability of the factors accounts for the variety of the lesions demonstrated.

Differential Diagnosis

Hemorrhages in the corpus callosum which are not traumatic in origin and resemble those shown in Figure 18 may be seen in encephalitis, severe toxic conditions, and fat embolism. Only the last is of real differential diagnostic interest, since it is usually a sequel of trauma, and hemorrhages in the corpus callosum may be caused by both trauma and fat embolism. Furthermore, the hemorrhages of fat embolism may be confined to the corpus callosum. Because of the great similarity of the two types of hemorrhages it is often impossible to differentiate them grossly. In trauma to the head even the histologic proof of fat in small vessels does not exclude the traumatic origin of at least some of the hemorrhages present. There is, however, a difference in the microscopic appearance of hemorrhages of the two types if they have developed in their characteristic pattern. In typical fat embolism there occurs first a plasma diapedesis from the obstructed vessel immediately proximal to the embolus. This is accompanied by hypoxic necrosis of the suffused nervous tissue. Then, from a more proximal portion of the obstructed vessel, an extravasation of red cells ensues whereby the red cells surround in shell-like fashion the plasma core, forming the characteristic picture of a ring hemorrhage. The brain tissue peripheral to the hemorrhage rarely becomes necrotic (Fig. 22). In a typical traumatic hemorrhage, however, all blood elements "bleed" at the same time. Consequently, a mixture of red cells and other blood elements accumulates around the lacerated vessel. Very often this hemorrhage or dysfunction of the traumatized vessel causes necrosis of nerve tissue surrounding the hemorrhage (Fig. 6). The structural difference of the two types of hemorrhages may be recognizable also in older cases in which the hemorrhage has been absorbed. In typical ring hemorrhage of fat embolism, glial proliferation is found in the formerly necrotic central area. This is surrounded by iron-storing phagocytes. In the old, traumatically-induced hemorrhages, in contrast, the iron is frequently accumulated in the center of the lesion, often around a small vessel and encircled by a

glial scar which also may contain some iron pigment if the hemorrhage had penetrated the necrotic area diffusely (Fig. 10).

Foci of anemic necrosis of the corpus callosum resembling those observed in a few of our cases are rare. They may be seen as sequelae of severe and subacute shifting of one hemisphere toward the other due to a rapidly increasing space-consuming lesion, as has been shown (Fig. 20). Their non-traumatic nature can be assumed only if no other definite manifestations of trauma are present within the brain and the deformation of the brain structures is sufficiently severe as to interfere with circulation as in the case described.

Occasionally areas of non-hemorrhagic necrosis of the corpus callosum are seen in cerebral arteriosclerosis. They are often of the incomplete type with degeneration of certain tissue elements and persistence of others, as was usually seen in our cases. In arteriosclerosis, however, there are always multiple vascular alterations, so characteristic for this disease that no differential diagnostic problem exists in regard to these lesions.

Embolic and thrombotic softenings with necrosis of all tissue elements and involving the corpus callosum solely are extremely rare. Their borderline usually exceeds the limits of the corpus callosum and corresponds with the supply area of the occluded arterial branch of which the corpus callosum is only one part. Hemorrhagic reaction within the softened white matter does not occur in arterial encephalomalacia but is often an indication of participation of veins in causing the tissue necrosis. However, we know of no report of corpus callosum softening due to venous thrombosis without extension of necrosis into neighboring areas.

There is only one disease in which the most characteristic lesion is necrosis of a part or all of the corpus callosum. It was first described by Marchiafava and Bignami,[9] in 1903, and in the American literature by King and Meehan,[10] Nielsen and Courville,[11] and recently by Merritt and Weisman.[12] It is characteristic of this condition that the dorsal and ventral fibers of the corpus callosum are spared and that the slowly progressive degeneration may extend into the white matter of the hemispheres without necessarily causing cavitation. This rare disease occurs in those with chronic alcoholism, and is usually ascribed to unknown properties of crude Italian wine and gross dietary insufficiency. In some of our traumatic cases the lesions strongly resemble those characteristic of this disease, even to the preservation of the dorsal and ventral layers of the corpus callosum. They could, therefore, easily be misinterpreted. Conversely, their similarity and the

fact that blunt injuries to the head are common among persons with chronic alcoholism suggest the possibility of necrosis of the corpus callosum in this disease being mechanically induced, at least in some cases. That no traumatic lesions have been mentioned in the cases of Marchiafava-Bignami's disease reported thus far, does not necessarily contradict this suggestion, since, as we have demonstrated, the corpus callosum may very well be the predominant site of the damaging effect of the trauma and small, old, accompanying contusions in the cortex and elsewhere may easily be overlooked. In the case of corpus callosum defect in a chronic alcoholic patient, recently described by Seitelberger and Solms,[13] we have the strong impression that the lesion was traumatic. It is described as a groove-like defect in the midline of the dorsum of the corpus callosum, beginning at the knee and extending into the splenium where it eventually separated all fibers. Our assumption is supported by the considerable coincidence of alcoholism and trauma to the head in our series. In 12 cases the victim was definitely either in an acute state of intoxication at the time of the accident or was known to be chronically alcoholic. The actual number may be even higher since in many cases the clinical record was incomplete as to the pre-admission history. Deficiencies in past histories of our cases is also evident in regard to former trauma to the head, thus rendering it difficult for the pathologist to explain isolated defects of the corpus callosum, such as a defect of the ventral layer of the caudal two thirds of the corpus callosum which we found in a patient with angioma of a temporal lobe (Fig. 23), not included in Table I. The lesion was confined to the midline and was in an advanced state of organization. A rusty brown discoloration indicated that the necrosis had been hemorrhagic. The patient was 29 years old and had no vascular alteration which could have caused the lesion. The angioma, however, may have led to fainting spells or even epileptic seizures during which the lesion may have developed on a traumatic basis. The morphologic features of the lesion were consistent with a mechanical injury. However, trauma was purely hypothetic because of the inadequacy of the clinical history.

In another case, that of an old, mentally ill patient, we found a small cyst in the left half of the ventral portion of the corpus callosum (Fig. 24). The oval shape of the cyst was similar to that of a single hemorrhagic lesion in one case of our series (Fig. 3). The lesion was not pigmented and its wall was remarkably smooth and lined with an accessory pia. This indicates that it must have been very old. It actually was reminiscent of lesions found in infant brains. As has

been shown by Spatz,[14] immature necrotic nerve tissue is always removed faster and more completely than adult nerve tissue, thus leaving a smooth-walled porencephalic defect. Hence, the lesion may constitute an abortive form of so-called congenital corpus callosum defect. Undoubtedly, these defects are very often congenital malformations. In certain cases, however, definite signs of a developmental disorder are lacking and to date no reasonable explanation for them has been offered. The possibility of traumatic origin has not been mentioned, probably due to the fact that other traumatic alterations are missing and the frequent involvement of the corpus callosum in trauma is not generally recognized.

Clinical Significance

We were not able to assign any specific clinical syndrome to damage of the corpus callosum even in those patients who survived for a period of time. The multitude of accompanying traumatic lesions always caused complex clinical pictures. In this we confirm Carpenter and Druckemiller[15] in their recent comprehensive review of the subject.

Summary

In 51 cases, or 16 per cent of our series of cases with blunt mechanical trauma to the head, the corpus callosum was found to be damaged.

Pathologically, the lesions may be subdivided into hemorrhage, hemorrhage and necrosis, and anemic necrosis, depending on the duration of survival and the reaction to the injury. The extent of the lesions varied from involvement of the entire corpus callosum to small, single, traumatic hemorrhages or foci of necrosis confined to a circumscribed area. Only one case showed complete tearing. The lesions occurred with and without skull fracture.

The most plausible theory as to their pathogenesis is that they are caused by sudden stretching and shearing forces due to elastic deformation of the skull and brain at the moment of the impact. In all cases, the force was vertically directed.

The traumatic lesions of the corpus callosum may resemble certain necrotizing lesions of the corpus hitherto considered to be non-traumatic in origin.

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LEGENDS FOR FIGURES

FIG. 1. Case 47. Very small traumatic hemorrhage in center portion of splenium, developing a few minutes after accident.

FIG. 2. Case 17. Small, fresh, irregularly shaped, massive hemorrhage in midline of corpus callosum, surrounded by an area of fresh necrosis. Nissl's stain. × 10.

FIG. 3. Case 48. Three-months-old massive hemorrhage in midline of corpus callosum, surrounded by a zone of tissue in a state of organization. Nissl's stain. × 10.

FIG. 4. Case 41. Faintly visible late phase of a hemorrhagic, traumatic lesion in lower portion of right half of corpus callosum.

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FIG. 5. Case 2. Multiple, necrotic foci in corpus callosum with small, diapedetic hemorrhages in centers. Survival 3 days. Weil's stain. × 40.

FIG. 6. Case 2. Small, diapedetic hemorrhage in center of an area of fresh necrosis in a 3-day-old lesion in the corpus callosum. Weil's stain. × 120.

FIG. 7. Case 23. Forty-two-day-old, incomplete necrosis of ventral portion of corpus callosum with severe loss of myelin sheaths. Weil's stain. × 10.

FIG. 8. Case 23. Larger area of traumatic necrosis in corpus callosum undergoing organization. Increased cellular density in focal area. Nissl's stain. × 40.

FIG. 9. Case 23. Compound granular cells partially lined up along persisting tissue fibers in an area of necrosis of the corpus callosum in a state of organization. Nissl's stain. × 600.

FIG. 10. Case 23. Forty-two-day-old lesion in the corpus callosum. Diffuse distribution of iron throughout the lesion and local accumulation of the pigment around small vessels. Iron stain. × 40.

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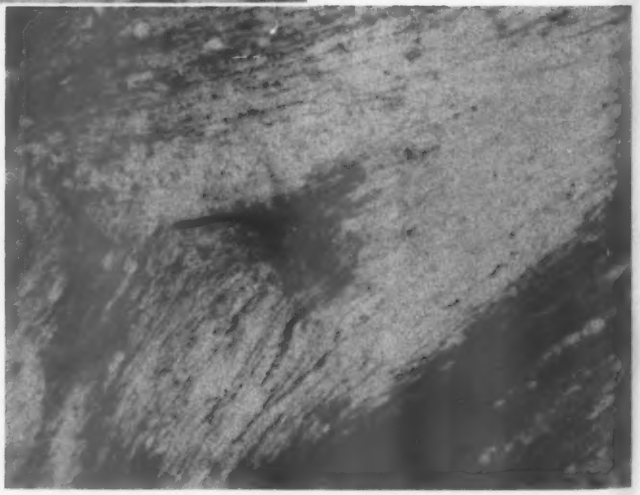

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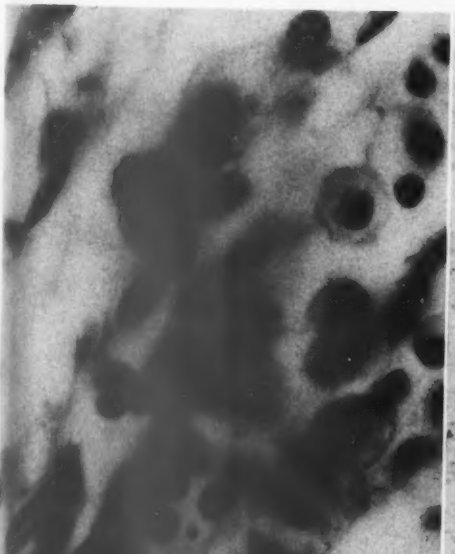

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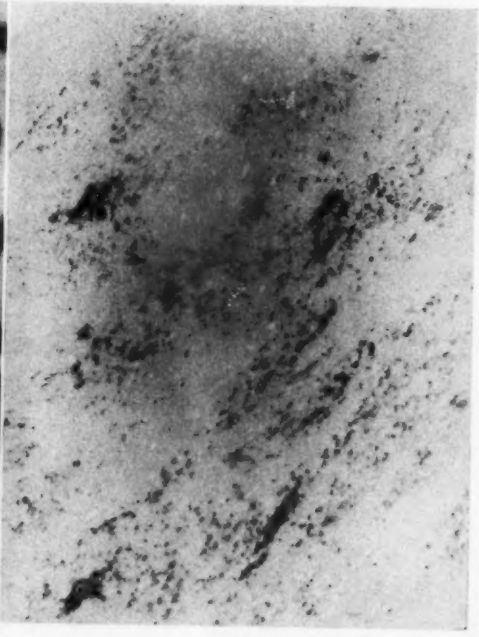

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FIG. 11. Case 49. Incomplete, traumatic necrosis of right half of corpus callosum and of fornices. Accompanying massive, traumatic hemorrhages in dorsal sections of both thalami.

FIG. 12. Case 2. Progressive reaction of glia and cellular elements of pathologically dilated vessel without hemorrhage. Leukocytes and individual phagocytes in dilated perivascular space. Nissl's stain. × 120.

FIG. 13. Case 5. Wedge-shaped, massive, traumatic hemorrhage in right half of corpus callosum accompanied by traumatic hemorrhages in cingulate gyri, septum pellucidum, right caudate nucleus, and adjacent internal capsule.

FIG. 14. Case 2. Severe traumatic lesion in the entire corpus callosum with hemorrhages and softening extending into white matter of cingulate gyri and septum pellucidum.

FIG. 15. Case 48. Diffuse degeneration of myelin sheaths in non-focal area of corpus callosum. Weil's stain. × 150.

FIG. 16. Case 48. First and second cortical layers of gyrus cinguli facing the sulcus callosus. Phagocytes in subarachnoid space. Glial cells of outer zone of the first cortical layer contain iron pigment, a residual of a 3-months-old, subarachnoid hemorrhage. Nissl's stain. × 112.

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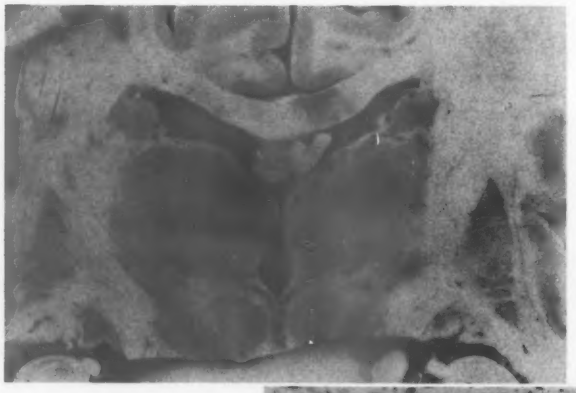

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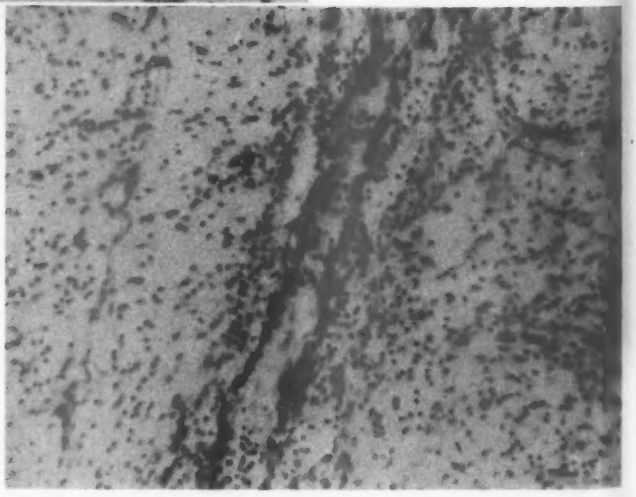

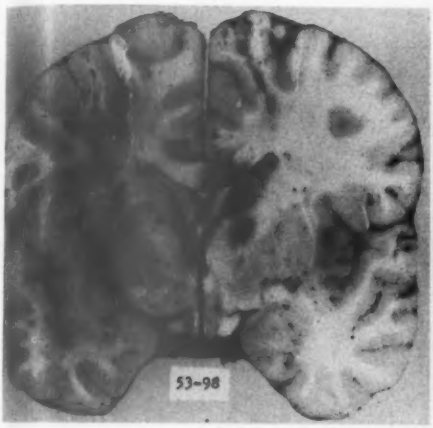

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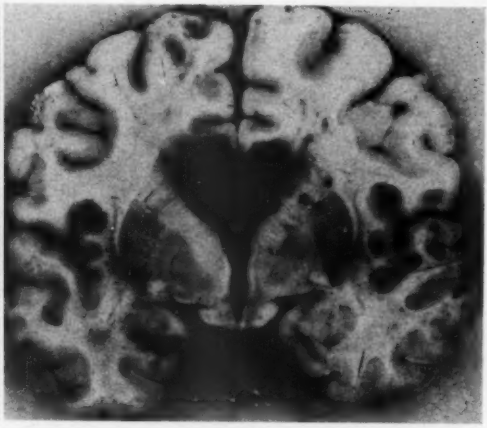

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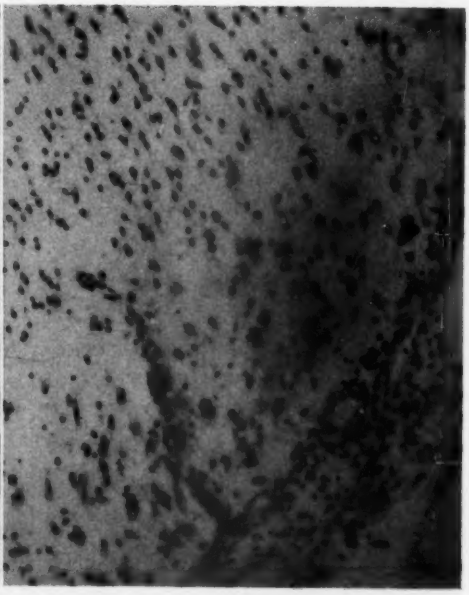

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FIG. 17. Case 1. Sagittal section through entire length of corpus callosum showing distribution of fresh, traumatic hemorrhages from knee to splenium. Secondary hemorrhages in midbrain and upper pons due to severe brain swelling.

FIG. 18. Case 6. Multiple, traumatic, individual, massive hemorrhages in center portion of corpus callosum. Traumatic destruction of left fornix, hemorrhages in right gyrus cinguli, right fornix, and left caudate nucleus.

FIG. 19. Case 3. Complete tearing of corpus callosum with necrosis of its stumps and hemorrhages in peripheral demarcation zones. The lesion extends into right cingulate gyrus. Destruction of septum pellucidum.

FIG. 20. Subacute shifting of right hemisphere toward left due to subdural hematoma, operatively removed. Pressure necrosis in right cingulate gyrus caused by falx and in right gyrus hippocampi caused by tentorium. Softening in left half of corpus callosum due to pressure by herniated right cingulate gyrus.

FIG. 21. Case 26. Traumatic, non-hemorrhagic, well demarcated softening in center portion of corpus callosum. The defect in the center of the softening is a post-mortem artifact.

FIG. 22. Fresh ring hemorrhage caused by fat embolism. Hematoxylin and eosin stain. × 160.

FIG. 23. Incidentally found, old, rusty brown defect of ventral portion of middle third of corpus callosum, possibly traumatic in nature.

FIG. 24. Incidentally found cyst in corpus callosum, possibly due to trauma during infancy.

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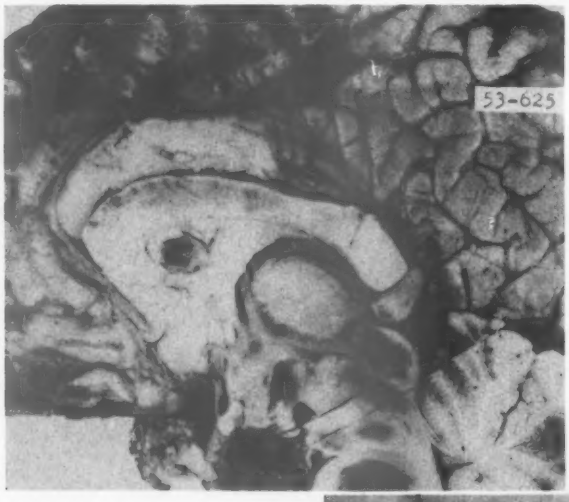

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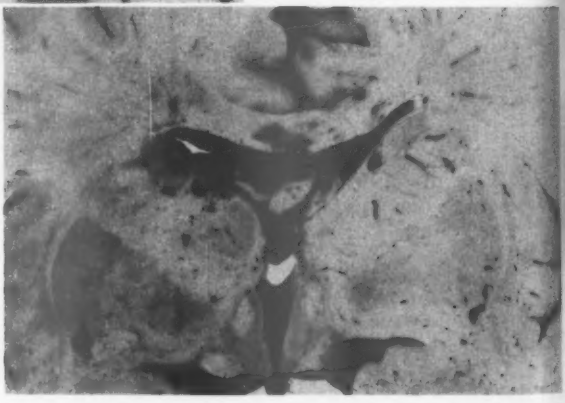

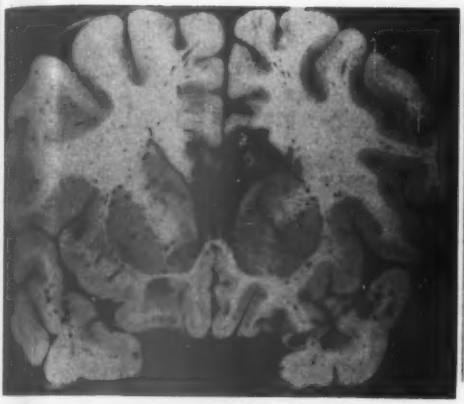

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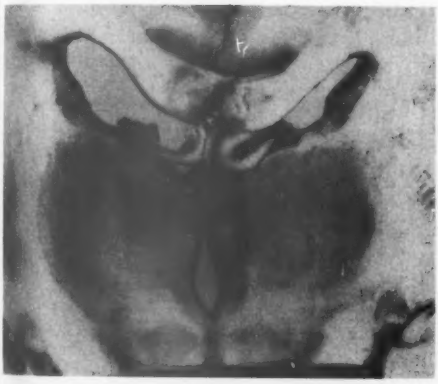

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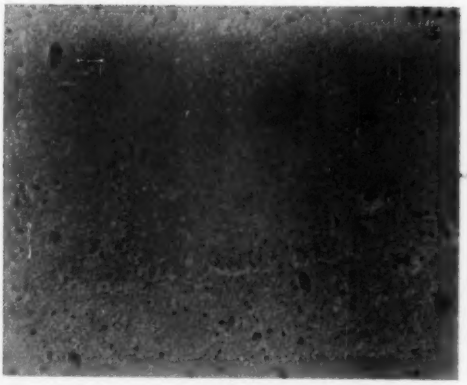

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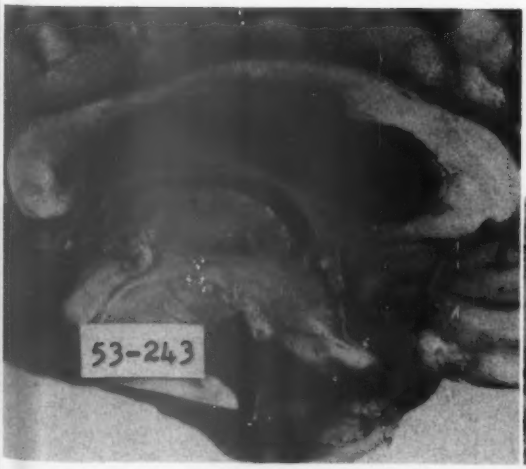

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HISTOPLASMOSIS OF THE CENTRAL NERVOUS SYSTEM *

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Through the years histoplasmosis has been a subject of great interest in the clinical and preclinical departments of Vanderbilt University Hospital. A recent case of histoplasma meningitis and the paucity of reports referring to lesions of the central nervous system in this disease prompted us to review our necropsy records. During the years 1925 to 1953, 23 cases of this disease were necropsied. The first case, discovered in 1932, was reported by Dodd and Tompkins.[1] Examination of the brain was performed in 11 of these 23 cases. Specific lesions involving the parenchyma of the brain, or the meninges or both, were found in 6 cases. These results bear out the prediction of Duarte[2] and Schulz[3] that a higher incidence of lesions of the central nervous system would be found if the brain were examined carefully in all instances.

Darling,[4] in his first case reports, described no lesions of the brain. After a review of the relevant literature Schulz[3] (1953) was able to find only one case of meningitis attributed to *Histoplasma capsulatum* (Duarte,[2] 1945). Although passing reference had previously been made to cerebral lesions in isolated reports, no detailed description of the lesions was included.[5-9]

Duarte's[2] case was one of meningitis due to *H. capsulatum* in a 23-year-old female domestic, who expired a few days after the onset of an acute illness characterized by headache, fever, nausea, and vomiting. No details were presented regarding the cerebrospinal fluid. At necropsy there were lesions of histoplasmosis in the ileum and purulent meningitis. The meningeal exudate consisted of both mononuclear and polymorphonuclear cells. Intracellular organisms typical of *H. capsulatum* were found in the meningeal and intestinal exudates.

Schulz,[3] in 1953, reported a second case of meningitis. This was in a 14-year-old boy who had been ill for about 1 month with fever, abdominal cramps, and vomiting. At necropsy there was disseminated histoplasmosis. The brain was not remarkable grossly except for convolutional flattening and clouding of the meninges between the cerebral peduncles. Microscopically, there was leptomeningitis which was more pronounced about the meningeal vessels. Although the vessel walls

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were described as intact, they were surrounded by a cuff of mononuclear macrophages, epithelioid cells, and some polymorphonuclear leukocytes. The specific parasites were found within the macrophages. Schulz reported also, as another instance of histoplasmosis of the central nervous system, the case of a 2-year-old boy which he encountered while reviewing histologic sections of necropsy cases of histoplasmosis. Parasite-laden macrophages were seen in the capsule of the pituitary gland and in the dura near the sella turcica. The pituitary gland was normal. No other sections of the brain were available.

The following 6 cases of histoplasmosis of the central nervous system illustrate the variety of lesions found in our material. Where possible, an attempt has been made to indicate the clinico-pathologic correlation and the relation of the intracranial lesions to those encountered elsewhere.

Report of Cases

Case 1 (V–47–147)

A 7-months-old Negro male was admitted to the hospital after an illness of 1 month characterized by fever, pallor, irritability, and weight loss. On examination there was fever of 100.8° F., pallor, dull expression, weak cry, enlarged posterior cervical lymph nodes, hepatosplenomegaly, and normal neurologic findings except for slight stiffness of the neck. There was an anemia, and histoplasmin skin tests were negative. *H. capsulatum* was cultured from the bone marrow and demonstrated in a marrow smear. The cerebrospinal fluid contained 3 to 5 leukocytes per cmm., 26 mg. per cent protein, and 44 mg. per cent sugar. The infant became progressively worse, with fever as high as 103.8° F. and increasing irritability. He expired on his ninth hospital day.

Necropsy. The characteristic lesions of acute disseminated histoplasmosis were present at necropsy. The usual involvement of the lungs, spleen, liver, lymph nodes, and bone marrow was encountered and typical organisms were present in large numbers in these organs. *H. capsulatum* was cultured from the spleen, liver, mesenteric lymph nodes, and the blood stream.

The cerebrospinal fluid was clear. The brain weighed 720 gm. and was generally soft. There was no flattening of the convolutions or meningeal exudate. On section no focal lesions were observed. The ventricular system was normal.

Microscopic Findings. The small meningeal and cerebral veins were intensely congested. In the subarachnoid space, especially over the cerebral cortex, there were appreciable numbers of large mononuclear phagocytes and some small lymphocytes. Occasional intracellular yeasts were seen in the large phagocytic cells. In addition, there was a very distinctive lesion in some sections of the cortex and pons. This

consisted of focal accumulations of large phagocytic cells just beneath the intima of small veins. These may be referred to as tubercles, although giant cells were not present and there was no caseation. Occasional lymphocytes and neutrophils were seen. The lesions were paravascular in location, encroaching upon the Virchow-Robin space and distorting the vessel wall (Fig. 2). The characteristic organisms were present in some of the macrophages. No parenchymal involvement was evident. The focal nature of the process was well demonstrated in longitudinal sections of vessels (Fig. 3).

Comment. This was the first (1947) lesion of the central nervous system found in cases of histoplasmosis necropsied at Vanderbilt University Hospital. Most conspicuous findings were the paravascular miliary granulomas just described. With respect to their focal nature and tubercle-like character they were strikingly similar to the unit lesions of miliary tuberculosis, although there was no caseation or giant cell formation. In addition to the focal granulomas, there was a mild meningitis manifested by slight exudation of large phagocytic cells and lymphocytes into the subarachnoid space.

Case 2 (V–48–185)

A 7-months-old white male was admitted to the hospital because of an illness of 5 months, characterized by pallor, intermittent low-grade fever, fluctuating cervical lymphadenopathy, and progressive distention of the abdomen. There was fever of 102.4° F., shotty cervical and axillary lymphadenopathy, pallor, hepatosplenomegaly, and normal neurologic findings. The patient was anemic. Late in the course of his illness *H. capsulatum* was demonstrated in bone marrow smear and culture. The cerebrospinal fluid contained 50 leukocytes per cmm., 19 mg. per cent protein and 39 mg. per cent sugar. Laparotomy was performed, and biopsies of the liver and mesenteric lymph nodes revealed *H. capsulatum* on microscopic section and culture. Coincidental with actidione therapy, skin sensitivity was lost. The child's clinical condition steadily deteriorated, and he expired 4½ months after admission.

Necropsy. The clinical diagnosis of generalized acute histoplasmosis was confirmed at necropsy. The usual involvement of the spleen, liver, lymph nodes, and lung was noted, along with numerous gastrointestinal ulcerations due to *H. capsulatum,* which was cultured from the lungs and liver.

No cerebral edema or meningeal exudate was present. On multiple sectioning of the brain there were no focal lesions and the ventricles were normal.

Microscopic Findings. There was diffuse engorgement of the small leptomeningeal and cortical vessels. In the subarachnoid space there was a mild increase in large mononuclear phagocytes clustered around congested small vessels. Typical intracellular organisms were found

without difficulty. Careful search of numerous sections failed to reveal granulomas within the brain substance.

Case 3 (V–52–65)

A 6-months-old white female, previously admitted twice because of a heart murmur and bronchopneumonia, was readmitted because of high fever. Splenomegaly was noted but no etiologic diagnosis was made. One month later she was again admitted because of persistent symptoms and further enlargement of the spleen. There was fever of 102° F., pallor, lethargy, a loud systolic heart murmur, hepatosplenomegaly, and an umbilical hernia. Neurologic findings were normal. There was anemia, and skin tests and complement-fixation tests for histoplasmosis were negative. The organism was demonstrated in specimens taken for biopsy from the spleen, liver, lymph nodes, and bone marrow. The disease progressed relentlessly, and the infant expired about 6 weeks after the final admission. Shortly before death the complement-fixation test for histoplasmosis became positive.

Necropsy. The anatomical findings were typical of generalized acute histoplasmosis. A congenital pulmonary valvular stenosis was present. *H. capsulatum* was grown from the lung and liver.

The brain weighed 550 gm. and was extremely congested. A few fine, tenuous meningeal adhesions were noted at the base of the brain. No evidence of active meningitis was seen. Sectioning of the brain disclosed slight dilatation of the lateral and third ventricles, apparently due to obstruction of the foramina of Luschka and Magendie. No other gross lesions were found.

Microscopic Findings. There was a mild meningitis characterized by infiltration of mononuclear cells and occasional lymphocytes and neutrophils (Fig. 4). There were no lesions within the substance of the brain. Typical intracellular yeasts were found in the meningeal exudate.

Comment. This case and the previous one illustrate a mild involvement of the meninges in association with disseminated histoplasmosis. No gross exudation was found in either case. Interestingly enough, in the second case there was dilatation of the lateral and third ventricles. Any attempt to explain this on the basis of granulomatous inflammation due to *H. capsulatum* in the region of the foramina of the fourth ventricle is speculative as microscopic sections were not obtained specifically from this area.

Case 4 (V–49–176)

A 5-months-old white male was admitted to the hospital because of a history of irritability, fever, anemia, and splenomegaly for 3 months. *H. capsulatum* had been cultured from the bone marrow at another hospital. There was fever of 101.4° F., pallor, lethargy, tachycardia, and hepatosplenomegaly. Neurologic examination showed no abnormalities. Anemia and leukopenia were present with a marked shift to the left. Skin tests and complement-fixation tests were negative for histoplasmosis. Blood and bone marrow cultures were positive. Despite treatment with anti-

biotics and ethyl vanillate, spiking fever and progressive weakness continued. The infant developed thrombocytopenia with widespread petechial hemorrhages and expired 1 month after admission.

Necropsy. The usual picture of acute disseminated histoplasmosis was encountered at necropsy. The organism was cultured from several organs.

Neither vascular engorgement nor meningeal exudation was noted. Multiple sections revealed no focal lesions, and the ventricular system was normal.

Microscopic Findings. There was mild meningitis manifested by vascular engorgement and small numbers of mononuclear phagocytes. In association with one small vein in the subarachnoid space, there was a solitary perivascular granuloma composed of large mononuclear cells without associated necrosis. There was a similar lesion in a perivascular space just beneath the ependyma of the lateral ventricle (Fig. 1). The choroid plexus was the site of perivascular granulomas and of diffuse mononuclear exudate in which intracellular organisms were found.

Comment. Here again we encounter focal perivascular miliary granulomas in the meninges, within the brain, and in the choroid plexus. There was an accompanying mild meningitis.

Case 5 (V–49–72)

A white male, 41 years old, was admitted to the hospital with a history of recurring cough, chest pain, fever, anorexia, weight loss, and hepatosplenomegaly for 9 months. Prior to admission *H. capsulatum* was demonstrated by smears and culture of a tonsillar ulcer. Four months before admission he developed left facial paralysis which subsequently cleared. There was right exophthalmos. He had low-grade fever and was cachetic. The other significant findings included ulcers of the tongue and tonsils, diminished breath sounds and tactile fremitus, and hepatosplenomegaly. On neurologic examination there was slight horizontal nystagmus, right exophthalmos, generally over-active reflexes, ill-sustained ankle clonus, and absent plantar responses. A mild anemia was found and histoplasmin skin tests were negative. Positive cultures were obtained from blood, sputum, bone marrow, and stool. No lumbar puncture was done. He grew progressively worse and died following a generalized convulsion 9 days after admission.

Necropsy. There were nodular, caseating lesions throughout the lungs at necropsy. Similar lesions involved and largely replaced the adrenal glands. Numerous ulcers of the large and small bowel were observed. Though the spleen and liver were enlarged, they were free of focal lesions. *H. capsulatum* was cultured from the lungs, spleen and blood, but it is noteworthy that the organisms were not found in routine histologic sections of the bone marrow, liver, or spleen.

The meninges were thin and transparent. The spinal fluid appeared clear and colorless, and the convolutions were not flattened. Numerous focal areas of softening up to 1 cm. in diameter were found on

coronal section of the brain; these were scattered throughout the white and gray matter of both hemispheres, principally in the frontal and temporal lobes. The pons, cerebral peduncles, and cerebellum were the sites of similar lesions; none was found in the medulla or upper cervical cord. The lesions were small and discrete, with a maximum diameter of 1 cm. Each was globular with a pale gray or yellow center which, though appearing soft, was firm to palpation. External to this was a darker gray zone which was quite distinct and peripherally faded into the normal brain tissue. Approximately thirty of these lesions were found. The ventricular system was normal and the choroid plexus was not remarkable.

Microscopic Findings. There were lesions of two types, with necrosis the main distinguishing feature between the two. The greater number were characterized by a central core of coagulative necrosis surrounded by a well delineated zone of large mononuclear cells containing innumerable *H. capsulatum* (Fig. 10). A few consisted simply of focal aggregations of large mononuclear cells and lymphocytes. There was no significant fibrosis or gliosis in association with any of these lesions. Moderate vascular engorgement was the only change in the surrounding tissue. These may be considered the unit lesions of the process, with the larger lesions apparently formed by coalescence of the microscopic tubercle-like structures (Fig. 9). Although the process extended into the cortex, there was no meningeal reaction.

An artery adjacent to one of these necrotic zones exhibited fibrinoid necrosis of its wall (Fig. 8). There was a pronounced perivascular reaction characterized by innumerable polymorphonuclear leukocytes. This type of inflammatory cell was very scarce elsewhere in the exudate. This may be considered as a primary vascular lesion although the possibility exists that the necrotic artery resulted from the adjoining inflammatory reaction. The resemblance between this vascular lesion and those noted in the meningeal vessels in case 6 is impressive.

Comment. In this necropsy we find a different picture as to distribution of lesions. This case does not represent the characteristic fulminant type of disseminated histoplasmosis commonly seen in childhood but, rather, a chronic and progressive type of focal involvement of different organs including the lungs, intestine, adrenal glands, and brain. In view of the extensive parenchymal disease the lack of meningitis is of interest.

Case 6 (V–53–229)

A white male, 55 years old, was admitted to the hospital with a history of productive cough, weakness, and lethargy for 6 weeks, and hiccup, dysarthria, and periods of disorientation for 10 days. His temperature was 100° F. Lethargy, emaciation, axillary and inguinal lymphadenopathy, hepatomegaly, and clubbed fingers were

noted. Breath sounds were diminished to absent over the posterior lung fields. Neurologic examination revealed disorientation, dysarthria, mild ptosis of the left eyelid, and subjective diplopia without demonstrable extraocular muscle imbalance. The reflexes were normal and sensation was intact. The clear cerebrospinal fluid was under a pressure of 60 mm. of water. It contained 116 white blood cells (63 per cent neutrophils) and 235 white blood cells (90 per cent neutrophils), 90 and 108 mg. per cent of protein, and 10 and 16 mg. per cent of sugar on two examinations. Cultures of the spinal fluid, sputum, and bone marrow for *Mycobacterium tuberculosis* were negative. The bone marrow culture eventually became positive for *Histoplasma capsulatum* after death of the patient. The course consisted of a rapid, relentless progression of symptoms to coma and he expired on his eighth hospital day.

Necropsy. There again was focal organ involvement with nodular caseating lesions of histoplasmosis at necropsy. Cavitation was evident in the lungs, which were also the site of numerous infiltrative lesions. There was similar involvement of the adrenal glands and ulcers of the ileum and large bowel. The etiologic diagnosis in this case was established by cultivation of the organism from the lungs and by the presence of typical intracellular yeasts in the lesions. All attempts to demonstrate acid-fast bacilli by culture and guinea-pig inoculation in this and the previous cases yielded negative results.

The brain weighed 1350 gm. and the cerebrospinal fluid was clear. At the vertex of the brain there was a mild thickening of the leptomeninges along the course of the smaller vessels, and innumerable small, white lesions were seen in the meninges over the convexity of the brain; these resembled small tubercles. Thick, yellow, meningeal exudate was present at the base of the brain, most prominent about the optic chiasm. Multiple coronal sections revealed no lesions within the substance of the brain. The ventricular system was not remarkable.

Microscopic Findings. There was marked meningeal inflammation which was most prominent around the vessels. This process was most evident in sections of the brain stem (Fig. 5). Similar areas, though not so frequent, were seen elsewhere, including the cerebellum and frontal cortex. The character of the exudate varied considerably but the essential relationship to the vessels was the most constant feature. There were nodular accumulations of large mononuclear cells about some meningeal veins, and many of these cells contained typical organisms in their cytoplasm. The most severe involvement was seen in and about arteries, especially those in the meninges at the base of the brain. On transverse sections many of these arteries exhibited fibrinoid necrosis of a part or all of their wall (Fig. 6). On longitudinal section this lesion was seen to be focal with interposed segments of normal vessel. In many vessels the entire wall was smudgy and eosinophilic. Pyknotic fragments were scattered throughout the

coagulum. The surrounding collar of polymorphonuclear leukocytes and large mononuclear cells outlined the necrotic vessels. There were myriads of yeasts, both extracellularly and within large phagocytic cells (Fig. 11). Many transitional lesions were found.

The earliest evidence of involvement of a vessel was the presence of a ring of mononuclear cells in the adventitia. Oftentimes this was accompanied by a lifting of the intima and early necrosis of isolated fibers in the media (Fig. 7). Further progression of the process was manifested by increasing necrosis of the arterial wall with aggregation of polymorphonuclear leukocytes and eventual production of the full-blown lesion. Thrombosis of the affected vessels was not prominent. In a few, small masses of fibrin were closely adherent to the damaged intima. The parenchyma was not involved.

Comment. The meningitis seen in this case was of a distinctive and interesting type. The over-all similarity to tuberculous meningitis, both in the gross aspects and to a certain extent on the basis of the microscopic appearance, was quite evident. The basilar localization, with tendency to involvement of the optic chiasm, was a striking feature. As with tuberculous meningitis, the exudate seemed to be primarily related to small arteries and veins. In many instances there was an adventitial infiltration quite similar to that seen in the arteritis associated with tuberculous meningitis. Except for the presence of the organism it would be difficult to distinguish the two. However, in the case at hand there was a distinctive lesion not seen ordinarily in association with tuberculous arteritis. This consisted of fibrinoid necrosis of the arterial walls with an accompanying exudation of polymorphonuclear leukocytes. Such exudation was not seen in the absence of necrotizing arteritis, and it was reasonable to assume that the neutrophils represented a response to the local death of tissue.

The pathogenesis of this striking and widespread arterial lesion is not clear from an anatomical study. Somewhat similar lesions are seen in other disease states, but in each instance certain features of the microscopic picture serve to distinguish the necrotizing arteritis encountered in this case from the previously described varieties. For example, the focal and eosinophilic necrosis of the vessel wall noted here is characteristically present in cases of Rocky Mountain spotted fever,[10] but the lesion in the latter disease is apparently due to the presence of the infecting rickettsiae within the smooth muscle cells of the arterial wall. In our experience and that of others, cerebral arteritis does not occur in spotted fever, and large numbers of polymorphonuclear leukocytes are not seen surrounding the necrotic arteries

elsewhere in the body. *H. capsulatum* cannot be found within the smooth muscle cells of the arteries, though it is certainly possible that the arterial necrosis may be due to the presence of the yeast itself, or to toxic products elaborated by the organism. Perhaps the extensive necrosis of arteries is brought about by the same mechanism whereby necrosis occurs in other lesions of histoplasmosis, but the peculiar localization and fibrinoid character of the necrotic vessel walls argue against this possibility.

Certain similarities exist in the structural alterations seen in the cerebral vessels in this case and those found in periarteritis nodosa and in other examples of so-called allergic arteritis.[11] The fibrinoid character and focal nature are common to each, but again massive leukocytic exudation about the arterial lesion, so prominent in our case, is not seen in periarteritis nodosa. Furthermore, destruction of the vessel wall commonly precedes cellular exudation in the vascular lesions of periarteritis nodosa, whereas exudation of mononuclear cells occurred prior to necrosis of the vessel wall in the case under discussion. In case 5, a similarly affected artery was found in association with a granulomatous lesion of the brain parenchyma (Fig. 8). The occurrence of necrotizing arteritis in these two cases of histoplasmosis of the central nervous system is especially intriguing, as we have never encountered arterial lesions in other organs in an extensive experience with this disease.

Discussion

The cases presented illustrate the varied and relatively frequent involvement of the central nervous system in our series of cases of histoplasmosis. In reviewing the material, we were surprised by the high incidence of cerebral lesions when searched for carefully. There is an increasing awareness of the prevalence of histoplasmosis, and it is well to emphasize at this time such an important aspect of the disease which has previously attracted little attention.

Peterson and Christie[12,13] have emphasized the diverse picture which may result from infection of the human host by *H. capsulatum* and have classified the various types of infection with reference to clinical course, skin sensitivity to histoplasmin, complement-fixation tests, and radiographic evidence of the disease. In the most benign form, diffuse pulmonary calcification and histoplasmin skin sensitivity represent the only stigmas of infection. In marked contrast is the usual fatal case, frequently occurring in childhood, with a stereotyped clinical course characterized by hepatosplenomegaly, anemia, and rapidly fatal outcome. Necropsy of such a case will always reveal

widespread lesions of the lungs, liver, spleen, lymph nodes, and bone marrow. These authors have pointed out also that intermediate forms may be seen with focal involvement of particular organs or organ systems. The disease in the last group tends to be more benign though death may result from extension of such lesions. Cases 5 and 6 of our series would fall into this intermediate group whereas the remainder represent examples of the fulminant disseminated variety. The lesions of histoplasmosis of the central nervous system tend to fall into groups which correlate well with the general picture of the disease in particular cases.

Generally many analogies between tuberculosis and histoplasmosis may be made. The ability of *H. capsulatum* to mimic the action of the tubercle bacillus in the human host has long been recognized. For example, similar granulomatous inflammation, pulmonary calcification, and cavitation are frequently encountered in both diseases. The lesions of histoplasmosis which we have noted in the central nervous system tend to emphasize this resemblance of inflammatory reaction to the two organisms.

Classification of the lesions of histoplasmosis of the central nervous system shows that they naturally fall into categories analogous with those of tuberculosis:

1. *Miliary granulomas* with a constant relation to small veins may be seen in the brain, meninges, and choroid plexus (Figs. 1 to 3). This type of lesion is seen in association with the acute disseminated form of histoplasmosis. These granulomas resemble closely the unit lesions of miliary tuberculosis, and an over-all comparison between miliary tuberculosis and histoplasmosis of this type might well be made.

2. Focal destructive lesions of the brain parenchyma may be seen, as exemplified by our case 5, a patient with progressive lesions of many different organs (Figs. 8 and 9). These discrete areas of necrosis with surrounding infiltrations of mononuclear cells may be compared to tuberculomas, the classical focal lesion of tuberculosis of the central nervous system. The term *histoplasmoma* might well be used to describe this type of lesion and it is conceivable that such a lesion might attain sufficient size to suggest the presence of an intracranial neoplasm by virtue of its location, or by the production of increased intracranial pressure.

3. Histoplasma *meningitis* is well demonstrated in our case 6. The ability of the organism to produce meningeal inflammation varying from a mild cellular exudation (Fig. 4) with no recorded clinical components to a severe and lethal meningitis with a remarkable clinical and pathologic similarity to tuberculous meningitis is evident

(Fig. 5). The spinal fluid findings and clinical course, as well as the basilar meningitis, pseudo-tubercles dispersed in the sulci along the course of the vessels, and the perivascular exudation make for almost identical pictures in our case 6 and the usual case of tuberculous meningitis. Thus, *H. capsulatum* must be considered as one of the many possible etiologic agents in cases of meningitis with a low cerebrospinal fluid sugar, after infection with a pyogenic organism has been excluded.

There seems little possibility that primary infection of the meninges or brain with *H. capsulatum* may occur. In each of our cases there was widespread evidence of infection elsewhere than in the central nervous system. However, it is reasonable to assume that subsequently cases of involvement of the central nervous system by *H. capsulatum* may be found with only minimal lesions in other organ systems. That is, secondary infection of the meninges or parenchyma of the brain might well occur from a small, relatively quiescent focus of infection in the lungs. Such occurrence would be entirely comparable to cases of tuberculous meningitis with no clinical evidence of activity of the disease elsewhere in the body. In view of the occurrence of these lesions it should be concluded that careful clinical search for the presence of involvement of the central nervous system should be carried out in every case of histoplasmosis.

Summary

Lesions of the central nervous system were present in 6 of 11 cases of histoplasmosis.

Neurologic symptoms and signs were prominent in 2 of 6 cases with cerebral lesions.

A classification is presented which emphasizes the similarity of these lesions to those of tuberculosis of the central nervous system.

The clinical and pathologic findings in this series emphasize the necessity for considering the possibility of involvement of the central nervous system in every case of histoplasmosis.

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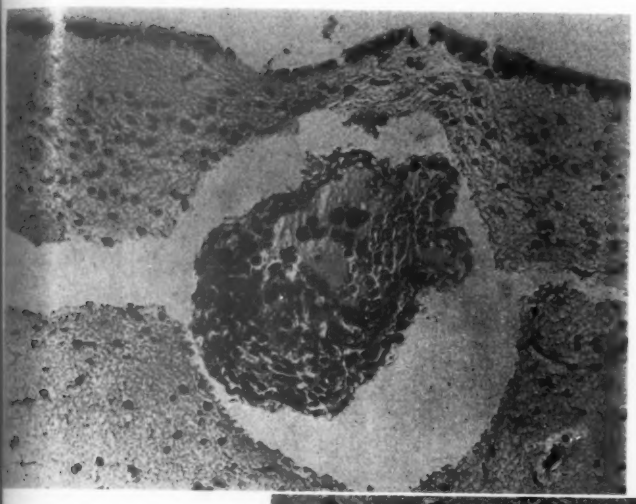
LEGENDS FOR FIGURES

All sections were stained with hematoxylin and eosin.

FIGS. 1 to 3. Representative miliary cerebral granulomas in disseminated histoplasmosis. The relation to the small veins and perivascular spaces is shown. *Fig. 1.* Case 4. Subependymal perivascular granuloma. × 145. *Fig. 2.* Case 1. Typical nodule composed of large mononuclear cells and lymphocytes. × 195. *Fig. 3.* Case 1. The relation of a granuloma to the long axis of a small vein (arrow) is shown. × 145.



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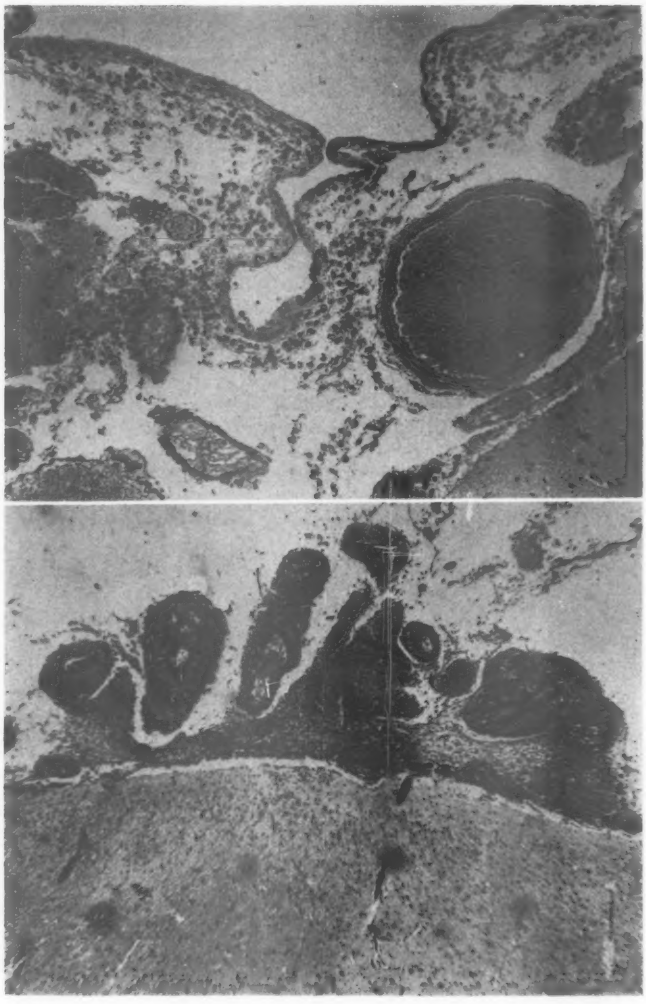
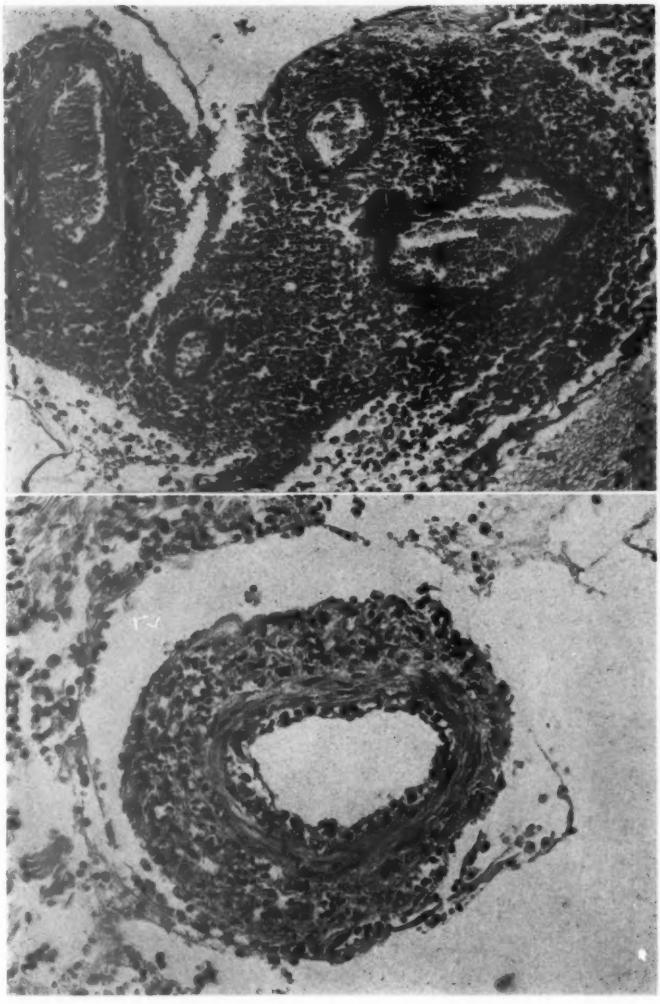
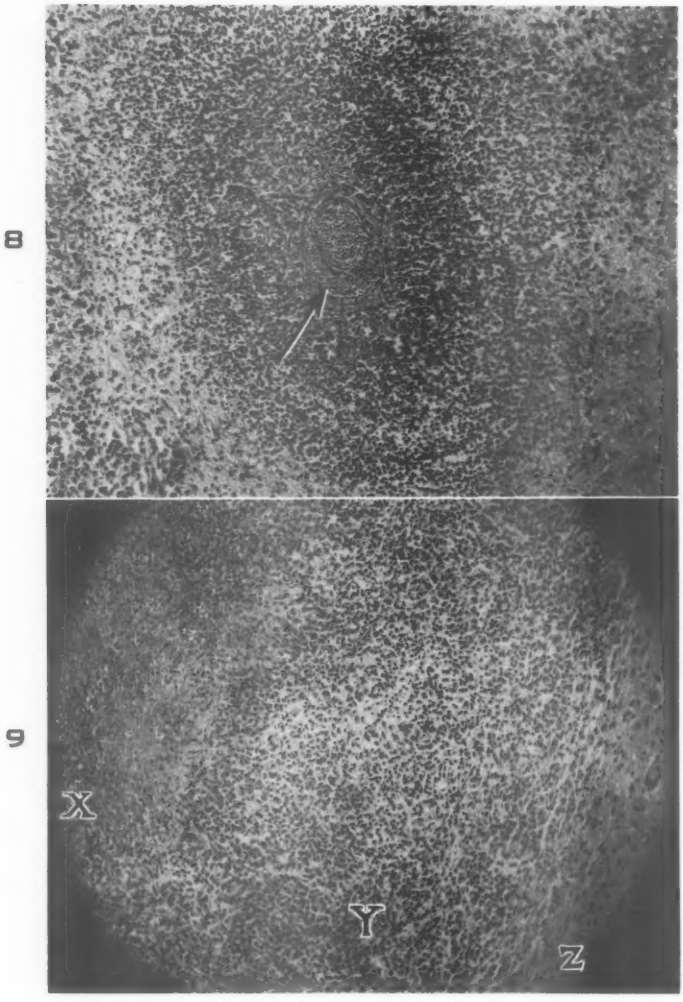


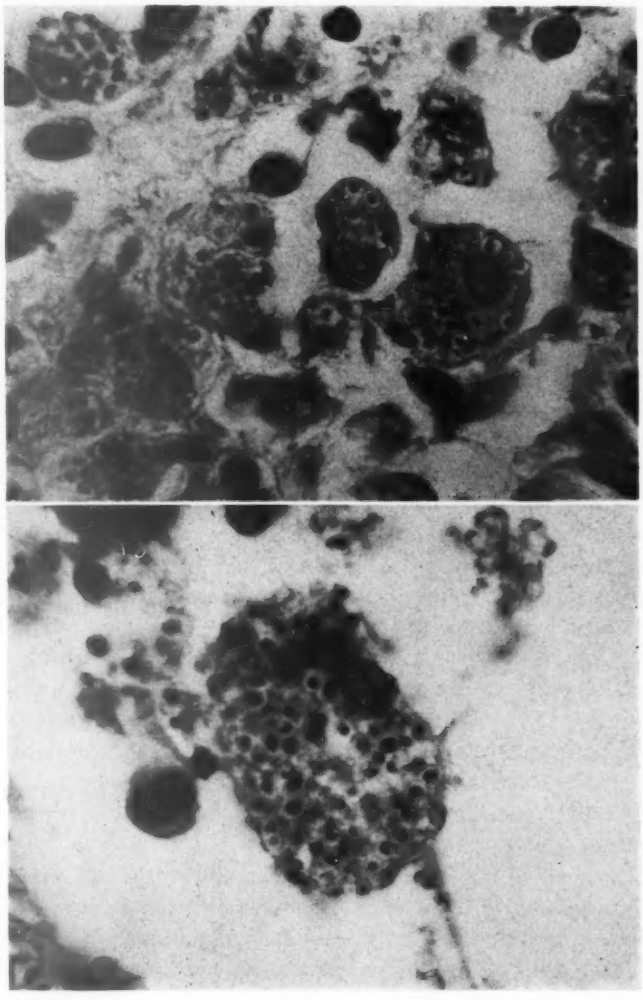
FIGS. 4 and 5. Meningitis in association with histoplasmosis. *Fig. 4.* Case 3. Mild reaction with small clusters of large mononuclear cells and lymphocytes in subarachnoid space. × 100. *Fig. 5.* Case 6. Marked exudation in subarachnoid space of mesencephalon. Relation of exudate to vessels is shown. × 50.



FIGS. 6 and 7. Arterial lesions in histoplasma meningitis. *Fig. 6.* Case 6. Focal fibrinoid necrosis of arterial walls with surrounding exudate of neutrophils and large mononuclear cells. × 125. *Fig. 7.* Case 6. Early arterial lesion with adventitial and subintimal exudation and intimal detachment. Isolated medial fibers are necrotic. × 200.



FIGS. 8 and 9. Parenchymal lesions of brain in histoplasmosis. *Fig. 8.* Case 5. Necrotic arterial wall (arrow) with collar of polymorphonuclear leukocytes. × 100. *Fig. 9.* Case 5. Focal parenchymal lesion with necrosis (X) and zone of large mononuclear cells (Y) adjacent to area exhibiting mild gliosis (Z). × 50.



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FIGS. 10 and 11. Typical *Histoplasma capsulatum* in cytoplasm of large mononuclear cells. *Fig. 10.* Case 5. There is an achromatic space about each organism. × 970. *Fig. 11.* Case 6. The variable but distinctive appearance of the organism is shown. × 970.

THE PATHOGENESIS OF FIBROCYSTIC DISEASE OF THE PANCREAS

Study of the Ducts by Serial Sections *

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In the past 15 years a large literature has accumulated concerning both the clinical and pathologic aspects of fibrocystic disease of the pancreas. Of the many theories which have been advanced to explain the pathogenesis, those which have achieved widest acceptance include: (1) deficiency of vitamin A, (2) imbalance of the sympathetic nervous system, (3) altered secretion of the glandular structures of the body, including the pancreas, (4) inflammation, and (5) congenital stenosis or atresia of the pancreatic duct system. We wish to review briefly the evidence in favor of the last of these.

A number of investigators have suggested that obstruction was present from the gross examination of the pancreatic ducts at necropsy (Parmelee,[1] 1 case; Andersen,[2] 4 cases; Benoit,[3] 1 case; Pugsley and Spence,[4] 1 case; Sprenger,[5] 1 case; Menten and Middleton,[6] 1 case (case 10); Sobel,[7] 1 case; Sako,[8] 6 cases). In reporting the pathologic findings in 87 cases of cystic fibrosis of the pancreas, Farber[9] stated that a definite narrowing of the pancreatic duct was present in an unstated, but small, number of cases, and that malformation of the pancreatic ducts was an occasional finding.

From histologic studies, but without doing serial sections, Livingston and Ferguson[10] demonstrated what they interpreted as stenosis of the main pancreatic duct, and Kaufmann and Chamberlin,[11] failure of proper development of the pancreatic duct system. In each instance, the report was based on one case.

Of considerably greater value in arriving at an understanding of the state of the pancreatic duct system are those studies in which the head of the pancreas and the papilla of Vater have been examined in serial sections. Kornblith and Otani[12] clearly demonstrated by serial sections the presence of stenosis of the main pancreatic duct in a case of meconium ileus. Oppenheimer,[13] by means of serial sections, observed atresia of Wirsung's duct in the duodenal musculature of a 10-months-old girl. Hurwitt and Arnheim[14] found stenosis of the duct

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of Wirsung in serial sections of the head of the pancreas in a case of meconium ileus. For their report of a case of cystic fibrosis of the pancreas with concomitant polycystic kidneys and polycystic liver, Norris and Tyson[15] studied the pancreas by serial sections and reconstruction of a portion of the pancreatic duct system. The main pancreatic duct could not be identified grossly or histologically. In serial sections of the larger ducts they noted that though there were "numerous zones of constriction between areas of dilatation, most of the ducts extended for considerable distances before ending blindly." These authors did not mention the disposition of the pancreatic ducts in the papilla of Vater.

Werthemann and co-workers,[16] in reporting on 19 cases of fibrocystic disease of the pancreas, examined the pancreatic ducts by serial sections in 7 cases. In 6 they observed that the main pancreatic duct split into numerous smaller ducts in the head of the pancreas. In most of their cases, the bile, cystic, or hepatic ducts, singly or in combination, were malformed. These authors suggested that fibrocystic disease of the pancreas is caused by maldevelopment of the pancreatic duct system.

In an earlier study of the pathogenesis of fibrocystic disease of the pancreas from this laboratory, Baggenstoss and co-workers[17] investigated 5 cases with the view of determining the patency of the main pancreatic ducts. Serial sections of the head of the pancreas, including the papilla of Vater, were examined. Of the 5 cases included in that study atresia of the main pancreatic duct within the papilla of Vater occurred in one case, atresia of the main pancreatic duct in the duodenal musculature in 2 cases, and atresia of multiple interlobular ducts in 2 cases.

Our report, consisting of 8 additional cases of fibrocystic disease of the pancreas, is a further attempt to investigate the status of the pancreatic ducts by means of serial sections of the head of the pancreas and papilla of Vater.

Materials and Methods

Eight additional cases of fibrocystic disease of the pancreas, 7 of which came from the files of the Section of Pathologic Anatomy of the Mayo Clinic, were found suitable for study.* In each of these cases serial sections were made of the head of the pancreas and the papilla of Vater. The sections were cut 10 μ in thickness and every

* One case was furnished through the courtesy of Dr. J. D. Kirshbaum, San Bernardino County Hospital, San Bernardino, Calif.

other one was mounted and stained with hematoxylin and eosin, numbered consecutively, and studied in series. The number of sections in each case varied from 170 to 734, with an average number between 400 and 500.

Five of the patients were boys and 3 were girls. The ages varied from 3 days to 11 months. Five of the 8 patients died in the first month of life, and 3 of the 5 had meconium ileus.

In the following discussion we propose to use the terms stenosis and atresia as defined by Patten[18]: "Any abnormal narrowing of a tubular structure (such as a blood vessel or a duct or a part of the digestive tract) is known as a *stenosis*. When the narrowing goes to the point of complete obliteration of the lumen, the condition is called *atresia*. These terms alone do not imply anything as to the causation of the condition they characterize."

Results

Atresia of the main pancreatic duct in the duodenal musculature was observed in one case of meconium ileus. In this case the main pancreatic duct was followed until its lumen had become greatly narrowed (Figs. 1 to 4) and finally could not be recognized. None of the other pancreatic ducts in this case was observed either to join the bile duct which emptied normally into the duodenum, or to empty itself directly into the duodenum. Within the pancreas numerous interlobular ducts extended for varying distances and ended blindly in the fibrous connective tissue.

In a second case of meconium ileus the main pancreatic duct within the papilla was moderately stenotic. The duct through the head of the pancreas was small (Figs. 5 to 7) and in the papilla became still narrower. Beyond the point of maximal narrowing the duct abruptly dilated to a normal diameter. The pancreas contained many large interlobular ducts which terminated blindly in the fibrous connective tissue.

In the remaining 6 cases, atresia of numerous interlobular and intralobular ducts was evident (Figs. 8 to 20). These ducts were often greatly dilated proximal to the point of atresia. Occasionally they were atretic locally, only to become patent again 10 to 20 μ beyond the point of atresia, and then to continue for a variable distance before ending blindly in the connective tissue. In some instances the ducts seemed to originate quite suddenly in the dense fibrous stroma, continue for varying distances receiving smaller ducts, and to end as abruptly as they began in the fibrous tissue. In all 6 cases of this

latter group the duct of Wirsung was patent throughout the head of the pancreas and the papilla of Vater, joining the common bile duct and subsequently emptying into the duodenum. In 3 cases Santorini's duct could be followed and was patent throughout its course.

Comment

The findings in these cases seem to bring additional supportive evidence to the contention that the fundamental defect in the pathogenesis of fibrocystic disease of the pancreas resides in the maldevelopment of the pancreatic duct system. To judge from our cases and those previously reported, the defect may involve either the main pancreatic duct (the duct of Wirsung), the interlobular ducts, or the intralobular ducts, singly or in combination. In 2 cases the smaller ducts within the pancreatic lobules seemed principally involved; in 4 cases the larger interlobular ducts were most conspicuously and most often affected, and in 2 cases the main pancreatic duct was involved as well as the interlobular ducts. It usually was not difficult to demonstrate atretic ducts, though in those cases in which the patient had survived for several months the changes were much more striking in that the ducts were more dilated and tortuous, and were surrounded by a thicker cuff of fibrous tissue. These ducts were easily followed through serial sections and usually terminated abruptly in the dense fibrous stroma. Sometimes a striking contrast was noted between the patent ducts and the atretic ones as they were followed simultaneously from section to section. The lumen of the patent duct was usually empty, the duct was lined by tall cuboidal or columnar epithelium, and the wall was thickened slightly or not at all by fibrous connective tissue. In contrast, the atretic duct was greatly dilated and tortuous, its lumen was filled with brightly staining eosinophilic hyaline material, often associated with cellular débris; the lining cells were flattened, or even absent in some areas. Surrounding the atretic duct was a thick cuff of fibrous tissue which was often fairly cellular.

In some instances we observed foci of chronic inflammatory cells, but these seemed unassociated with the regions of atresia.

At necropsy the pancreas was stated to be grossly normal in all 8 cases. No attempt had been made to probe or dissect the duct system. In all cases serial sections revealed a patent common bile duct which emptied normally into the duodenum. Werthemann and associates[16] described cases in which only a portion of the pancreas was involved in the fibrocystic process. We have not found other descriptions of similar cases, nor did any of our present cases conform to this pattern.

The process of maldevelopment in those cases of fibrocystic disease demonstrating abnormalities of the pancreatic duct system is difficult to explain. According to Arey,[19] the pancreas is formed from two outpouchings of the endodermal wall of the duodenum in embryos of 3 to 4 mm. These hollow endodermal buds arise from the dorsal and ventral sides of the duodenum and are known as the dorsal and ventral pancreases, respectively. The dorsal pancreas grows more rapidly and extends into the dorsal mesentery and the dorsal layer of the omental bursa. The ventral pancreas, arising slightly cephalad to the hepatic diverticulum, remains small. As the bile duct elongates, the ventral pancreas, separated from the duodenum by this process, comes to drain into the common bile duct. Owing to the unequal growth of the duodenal wall, the two pancreatic primordia approach each other and about the seventh week they coalesce. The two duct systems anastomose and eventually the long distal portion from the dorsal pancreas and the entire duct from the ventral pancreas together form the duct of Wirsung (ductus major). The proximal segment of the duct of the dorsal pancreas forms the accessory duct of Santorini (ductus minor). The acini form as terminal and side buds of the ducts.

During normal embryonic development of the duodenum and biliary duct system, proliferation of the lining epithelium results in a temporary occlusion of the lumen.[20] This process occurs in embryos of from 4.5 to 16 mm. Through a process of vacuolation the continuous lumen is restored. In an effort to ascertain whether a similar process occurs in the development of the pancreatic duct system, Hurwitt and Arnheim[14] examined serial sections of the region, including the pancreases of 54 embryos ranging from 3 to 48 mm. In 9 embryos ranging from 3 to 6.2 mm., pancreatic tissue was present but ducts could not be recognized. In 45 embryos varying from 6.4 to 48 mm., the pancreatic ducts were patent throughout their length. A similar study cited by these authors of embryos ranging from 4 to 50 mm. in the Harvard collection also failed to demonstrate occlusion of the pancreatic duct system. Hurwitt and Arnheim considered, however, that lack of an obliterative phase in the development of the pancreatic ducts did not exclude maldevelopment as the basis for the stenosis in their case and in that of Kornblith and Otani.[12]

Most authors who have investigated the genetic aspects of the disease are agreed that the malady has a firm basis in heredity. Andersen and Hodges,[21] Goodman and Reed,[22] and Lowe, May, and Reed[23] have stated that fibrocystic disease of the pancreas behaves as a sim-

ple recessive trait. In 2 of our 8 cases developmental anomalies were present. In one case we noted alveolar dysplasia of the lungs. In a second a 3-day-old baby had congenital bilateral hydronephrosis, hydro-ureter with no demonstrable obstruction of the urinary tract, and dysplasia of one kidney. A sibling of this child had died in the neonatal period because of maldevelopment of the intestine of an unstated variety.

Farber[9] has contended that "if congenital obstruction of ducts were the cause of the pancreatic fibrosis, meconium ileus should be a constant finding in all patients with this lesion, and such is not the case." Our observations do not support this thesis. In a previous study[17] by one of us, serial sections of the papilla of Vater and the head of the pancreas revealed congenital atresia of the duct of Wirsung at the outer edge of the duodenal muscle in 2 cases and atresia within the papilla of Vater in one case, but meconium ileus occurred in only one of these 3 cases. In Oppenheimer's case[18] of congenital atresia of the duct of Wirsung, there was no meconium ileus. Furthermore, evidence of the presence of meconium ileus has been observed in certain cases in which the pancreas is normal. Fanconi[24] described one patient (case 5) having congenital atresia of the bile ducts with meconium ileus but a normal pancreas. Landsteiner[25] described a similar case. There can be no doubt, however, that in most cases meconium ileus is closely associated with cystic fibrosis of the pancreas. The relationship of congenital atresia of the duct of Wirsung to the complex of pancreatic cystic fibrosis and meconium ileus can be ascertained only by careful examination of serial sections of the head of the pancreas and papilla of Vater.

It seems probable in the light of our findings and those of others, that in cystic fibrosis of the pancreas varying degrees of obstruction of the pancreatic duct system may be present. In some cases, probably a minority, as in instances of atresia or marked stenosis of the main pancreatic ducts, the pancreatic flow may be completely blocked. To judge from such cases in the literature (Kornblith and Otani,[12] Hurwitt and Arnheim[14]) and from one of our cases in which serial sections of the head of the pancreas have been examined, complete or nearly complete obstruction of the duct is, in fact, sometimes found in meconium ileus.

In other cases few or many of the interlobular and intralobular ducts may connect with the main pancreatic duct which is entirely patent. It seems possible, however, that the age of onset and the severity of the disease may be dependent on the degree of obstruction

present. We believe that the number of cases which have been examined with this in view is not sufficiently great to permit final conclusions. In every instance in our laboratory (13 cases) in which the papilla of Vater and the head of the pancreas have been investigated by serial section techniques, abnormality, either stenosis or atresia, of the pancreatic ducts has been found.

Summary and Conclusions

Eight cases of fibrocystic disease of the pancreas were studied by serial sections of the head of the pancreas and the papilla of Vater in an effort to determine the patency of the terminal pancreatic duct system.

In 2 cases the main pancreatic duct was found to be abnormal. In one of these cases the main pancreatic duct in the duodenal musculature was atretic. In the second case the main pancreatic duct was narrowed in the papilla of Vater. In both there was associated atresia of many interlobular ducts of the pancreas.

In 6 cases the main pancreatic duct was patent but many of the interlobular and intralobular ducts showed atresia.

The rôle of maldevelopment of both the large and small components of the pancreatic duct system in fibrocystic disease of the pancreas can best be ascertained by careful examination of the pancreas and its ducts by serial section techniques.

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LEGENDS FOR FIGURES

FIGS. 1 to 4. The main pancreatic duct (duct of Wirsung) was followed from the pancreas into the duodenal musculature through sections shown in Figures 1, 2, and 3. In the following serial block, of which Figure 4 is an example, the duct could not be recognized. Hematoxylin and eosin stain. × 60.

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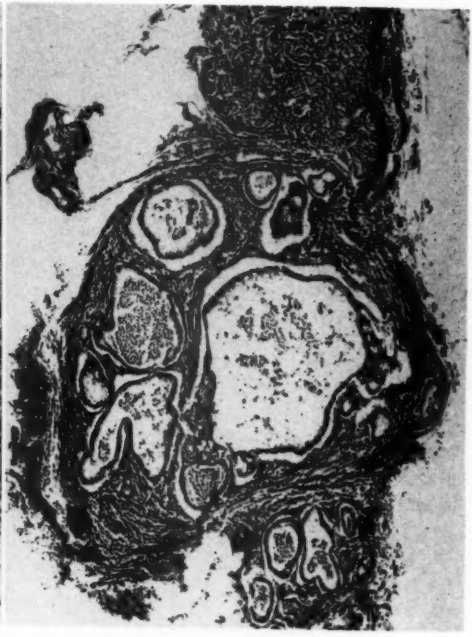

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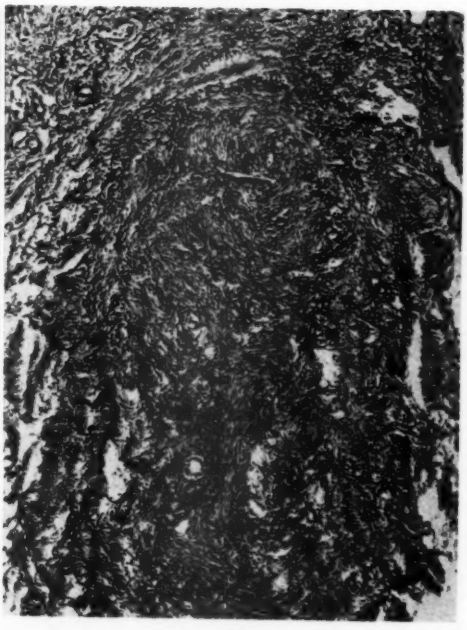

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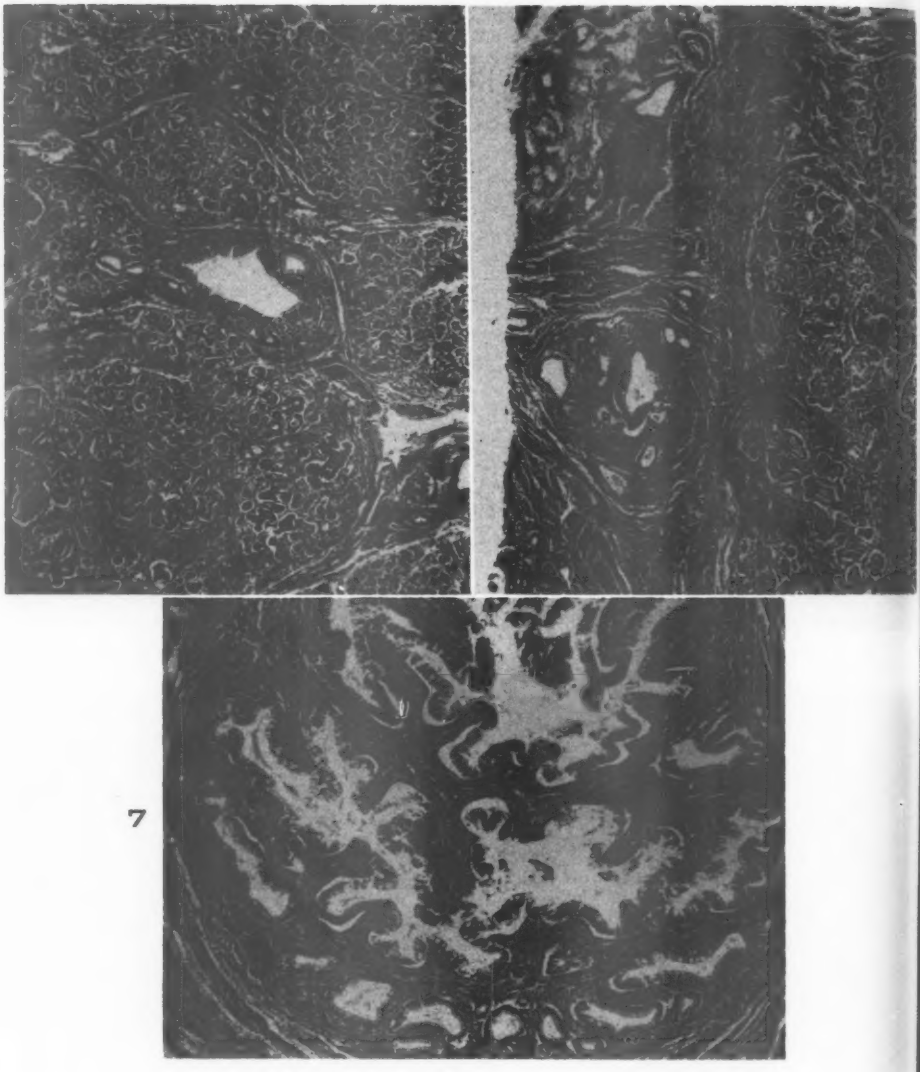

FIGS. 5 to 7. The duct of Wirsung was followed from the head of the pancreas (Fig. 5) into the duodenal musculature (Fig. 6) where it was narrowed. The duct dilated to normal size in the papilla (Fig. 7). The largest duct to the left in Figure 7 is the duct of Wirsung; that on the right is the common bile duct. Hematoxylin and eosin stain. × 55.

FIGS. 8 to 11. The sections illustrated in Figures 8, 9, 10, and 11 show evidence of atresia of a large interlobular duct. The duct ended blindly in the dense fibrous tissue. Hematoxylin and eosin stain. × 45.

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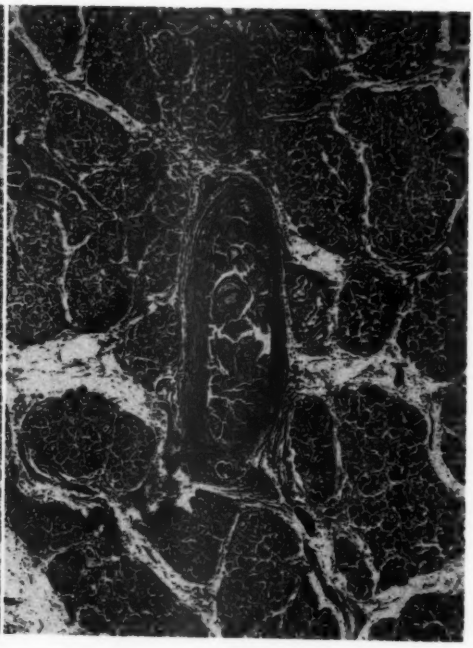

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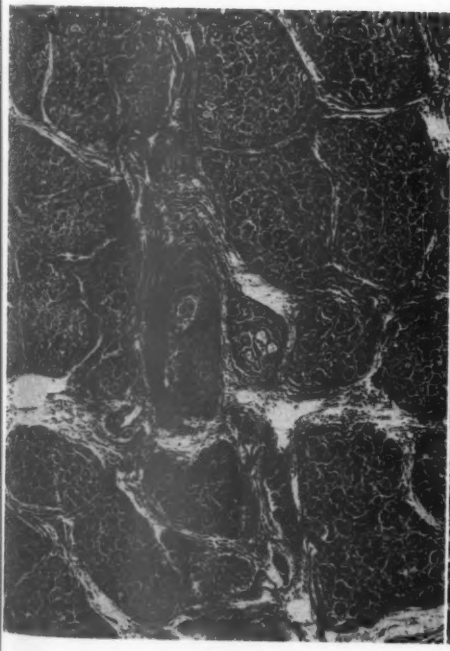

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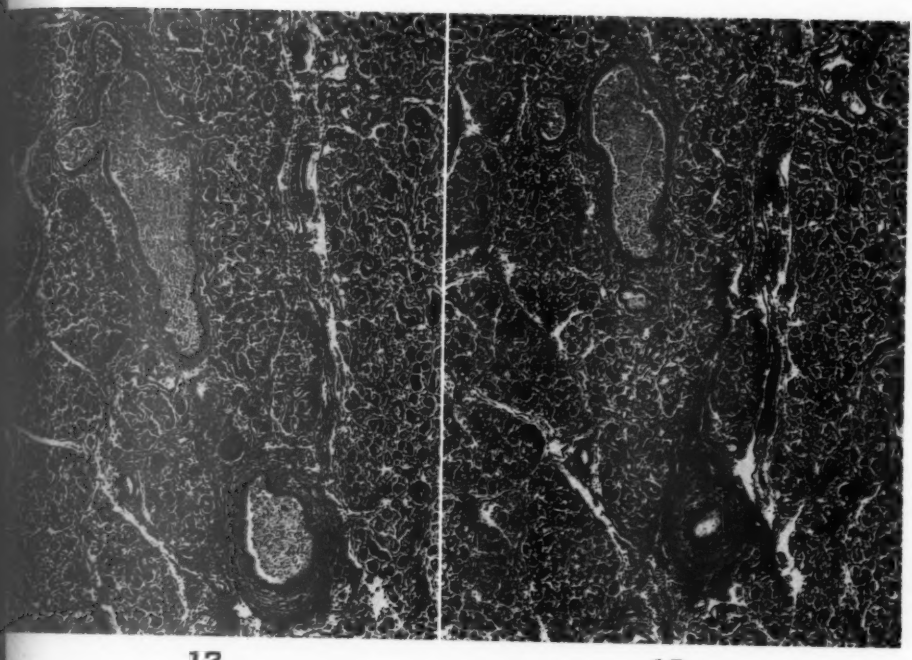
FIGS. 12 to 15. Two large ducts were followed simultaneously from the pancreas toward the duodenum through a distance of 5 consecutive sections, 4 of which are shown (Figs. 12, 13, 14, and 15, respectively). There was atresia of both ducts. Hematoxylin and eosin stain. × 55.



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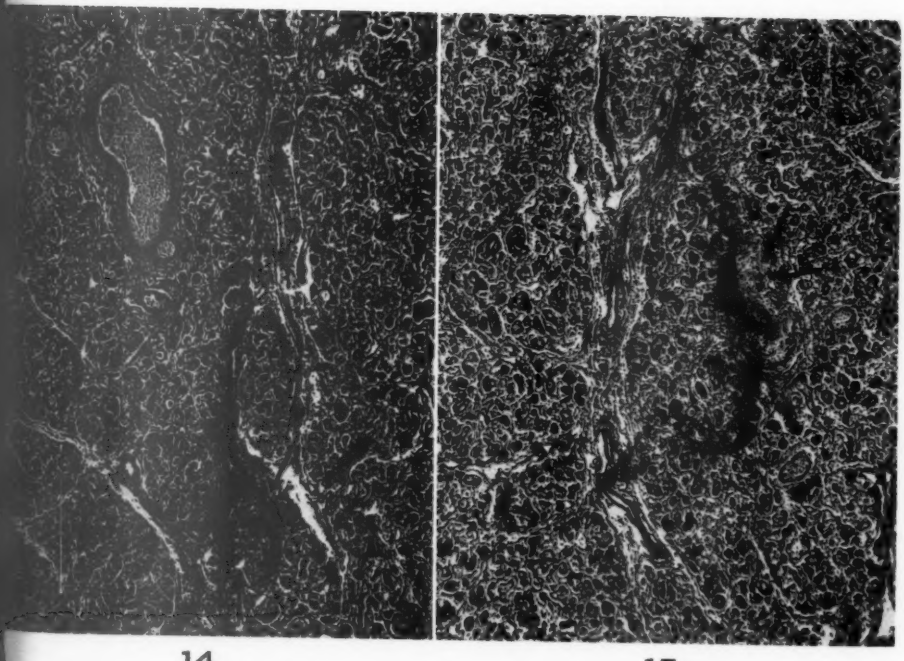

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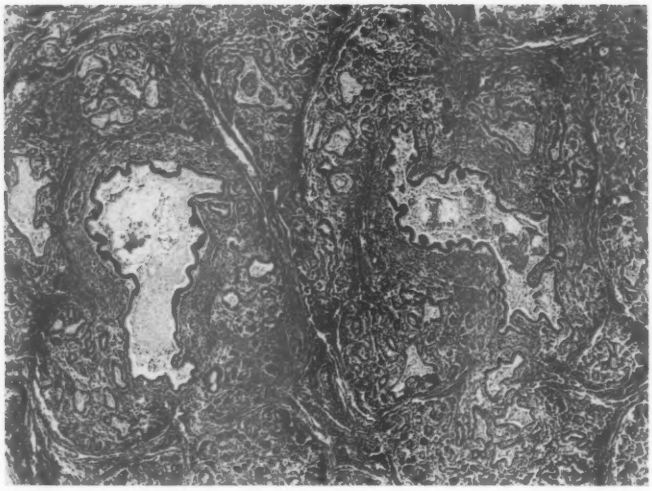
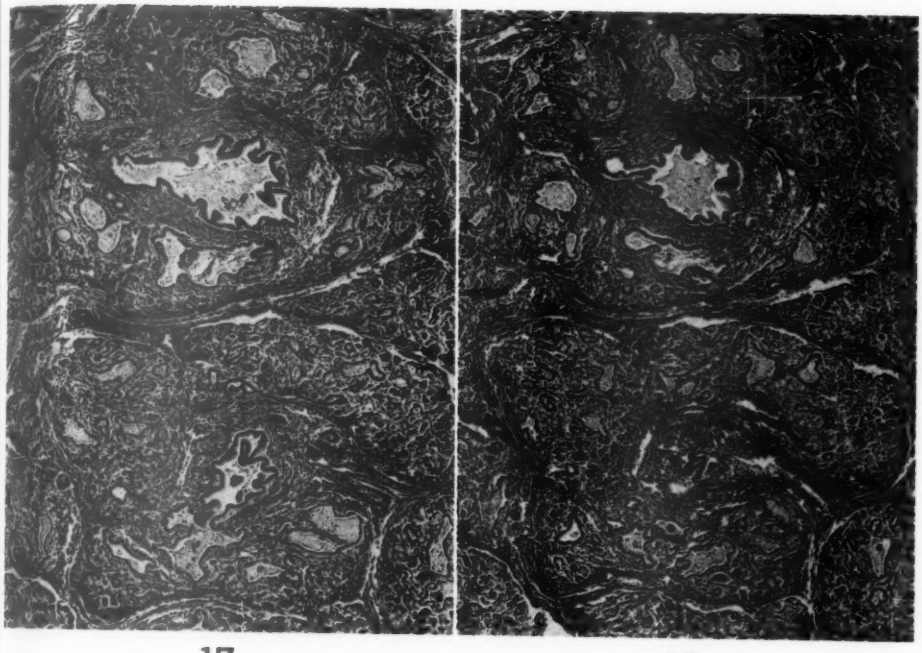


FIGS. 16 to 20. Two ducts were followed through sections shown in Figures 16, 17, 18, 19, and 20 from the pancreas to the duodenum. Both ducts ended in the fibrous connective tissue. Hematoxylin and eosin stain. × 45.



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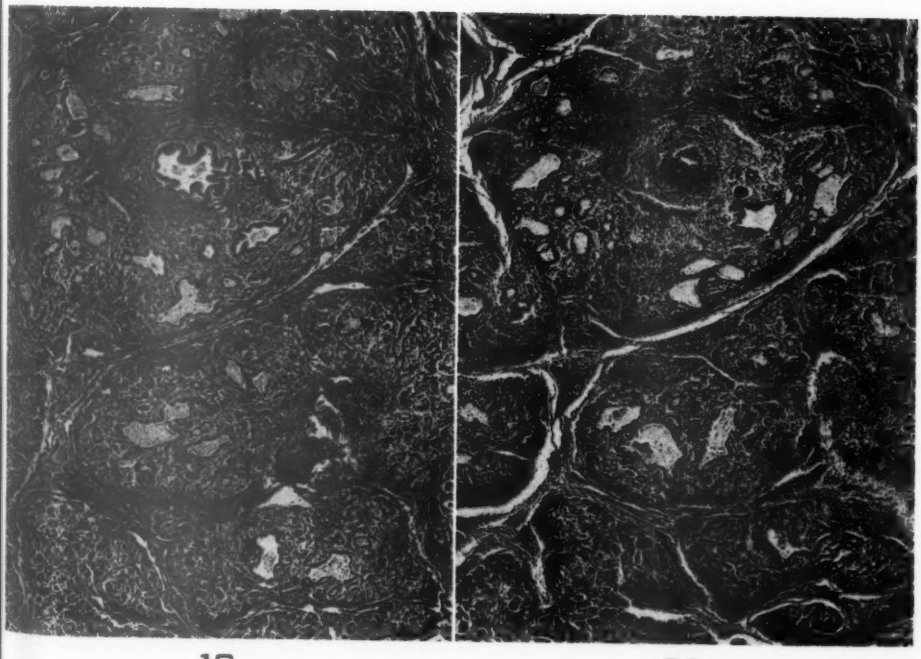

19 20

SUCCINIC DEHYDROGENASE ACTIVITY IN MYOCARDIAL INFARCTION AND IN INDUCED MYOCARDIAL NECROSIS *

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In experimentally induced renal necrosis, histochemically demonstrable succinic dehydrogenase activity in damaged cells is either reduced or disappears completely.[1] Since the heart contains large amounts of this enzyme, it seemed of interest to ascertain whether similar changes in enzymatic activity occur in myocardial necrosis.[2] This report deals with profound changes in the dehydrogenase activity in ischemic heart muscle cells which take place only a few hours after the onset of ischemia. Similar changes could be demonstrated also in the hearts of rats in which necrosis of the myocardium was induced by various drugs.

A marked decrease of microscopically demonstrable dehydrogenase activity was recently described in the heart as well as in other organs of rats following bilateral adrenalectomy.[3] Since the reported findings in the hearts were similar to those in myocardial necrosis, it was deemed advisable to repeat these experiments. However, no such decrease in enzymatic activity in adrenalectomized rats could be detected.

METHODS

Human Material

Blocks were taken from 10 hearts of persons with typical histories of acute myocardial infarction. Blocks were secured also from hearts which at necropsy showed evidence of rheumatic heart disease, and from the hearts of individuals who succumbed to various diseases with no gross or microscopic evidence of cardiac involvement. Necropsies were performed 2 to 15 hours after death. Fresh frozen sections, generally 10 to 15 μ thick, were cut without fixation on a Sartorius microtome. This microtome is equipped with a special cooling device for the cutting knife and was found to be very useful for the preparation of thin frozen sections suitable for histochemical staining.[4] Sections were dipped in tap water for a moment and then immersed in the incubation mixture for 1 to 2 hours at 37° C. under aerobic conditions as previously described.[1] Sections were then washed in tap water, immersed for several hours in 10 per cent formalin, and mounted

* Received for publication, July 10, 1954.

on glass slides covered with glycerin. The coverglass was rimmed with nail polish. Such preparations were usable for many months, if kept in the dark. Occasional sections were counterstained with hematoxylin. In addition, adjacent tissue blocks were fixed in formalin and prepared for routine sections.

Animal Experiments

Cardiac lesions were produced by means of fluoracetate[5] and plasmocid (8-[3-diethylaminopropylamino]-6-methoxyquinoline)[6]* in young male and female rats of the Wistar strain. Fluoracetate was given in a dose of 3 mg. per kg. of body weight intraperitoneally. The injection was repeated after 24 hours and the animals sacrificed 4 hours later. Several other rats received 2 doses of 2 mg. each at an interval of 1 hour instead of the 3 mg. dose. Plasmocid was injected intraperitoneally in 2 divided doses of 6 mg. each with an interval of 1 hour. The animals were sacrificed 22 to 24 hours later. A few animals were permitted to live 48 hours.

Larger rats were used for adrenalectomy. Both adrenal glands were removed under light ether anesthesia. A second group of sham-operated animals was used as controls. In these, the adrenal glands were exposed and the wounds afterwards closed. All animals were given normal saline solution to drink and food ad libitum. They were sacrificed after 4 days. Tissues removed and stained for dehydrogenase activity included the heart, kidney, liver, and brain. In general it was sufficient to leave sections of the heart for 10 to 30 minutes in the incubation mixture.

Results

Distribution in the Normal Human Heart

The adult human heart showed dehydrogenase activity only in muscle fibers (Fig. 1). Connective tissue, vessels, valves, endocardium, and pericardium remained unstained. The reduced tetrazolium was seen as rather coarse granules about 0.5 to 1.5 μ in diameter. The granules were arranged in parallel lines along the myofibrils. They covered the sarcoplasm almost completely, but the sarcoplasm remained unstained. Nuclei showed no activity.

Even in hearts from necropsies performed as late as 15 hours after death, satisfactory preparations could be obtained, although the formazan granules in these preparations sometimes were less intensely colored.

* Obtained from the Laboratory of Tropical Diseases, National Microbiological Institute, Bethesda, Md.

The Influence of Myocardial Infarction

Of the patients with acute myocardial infarction, one died 1½ hours; one, 2 hours; 3, 6 to 8 hours; one, 14 hours; and the remaining 4 patients, 36 to 140 hours after the onset of typical acute symptoms. In all cases, the coronary arteries showed severe sclerosis with marked narrowing. Fresh thrombotic occlusions were found in seven hearts. In all 5 cases in which the symptoms had lasted longer than 8 hours, typical, grossly recognizable infarcts were found. In the 3 cases in which the time interval between onset of symptoms and time of death was between 6 and 8 hours, areas of congestion and slight discoloration suggested the sites of infarction. In the hearts of the 2 individuals who died 1½ and 2 hours, respectively, after the onset of clinical symptoms, no distinct gross findings of acute necrosis were seen. All hearts, however, showed myocardial fibrosis to a varying degree.

In the heart of the patient who survived only 1½ hours, microscopic evidence of acute necrosis was not seen. There were, however, extensive areas of old fibrosis as well as foci in which granulation tissue had replaced dead muscle fibers. These areas were devoid of dehydrogenase activity. In some of the apparently undamaged muscle fibers in the vicinity of these abnormal areas, moderate reduction of dehydrogenase activity was seen.

In the heart of the patient who survived for 2 hours, routine staining showed only a few focal areas of myocardial necrosis. In these necrotic fibers dehydrogenase activity had completely disappeared. Reduction of enzymatic staining, however, was seen also in many muscle fibers that showed no unequivocal microscopic evidence of damage with routine stains (Fig. 2). In some of these the reduced tetrazolium was not deposited in the usual granular form but rather in irregularly shaped, larger particles. The cytoplasm often stained pink. In many muscle fibers with reduced deposition of dye, cross striation appeared entirely normal.

In the hearts from two patients who survived the acute attack for 6 to 8 hours, the changes observed were similar to those described. In the third case, however, necrosis of muscle fibers was extensive on routine staining and in addition there was considerable infiltration by polymorphonuclear leukocytes within the areas of necrosis. The necrotic fibers showed complete loss of enzymatic activity. It is probable that in this instance the onset of myocardial infarction antedated the clinical symptoms, since Mallory and his co-workers[7] have shown that diffuse infiltration by polymorphonuclear leukocytes begins after

about 5 hours but will not become extensive before 24 hours have elapsed.

In the remaining cases in which myocardial infarction had taken place 14 to 140 hours before death, there was extensive necrosis of muscle fibers with complete inactivation of the enzyme (Fig. 3). Deposition of dye was not noticed in polymorphonuclear leukocytes or in other inflammatory cells. In all of these hearts, areas of scarring, subacute fibrosis, and subacute myomalacia showed a complete lack of enzymatic activity. Within areas of subendocardial fibrosis in the hearts of these patients as well as in those with rheumatic heart disease, surviving muscle fibers showed a varying but often reduced degree of enzymatic activity (Fig. 4).

The Effect of Fluoracetate and Plasmocid on the Heart of the Rat

Following the use of fluoracetate and plasmocid, focal areas of myocardial degeneration were seen. These were often located beneath the endocardium. In general, damage was considerably more marked with plasmocid. In several rats that were sacrificed within 24 hours, the damage was discernible in preparations stained with hematoxylin and eosin by some increase in the eosinophilia of the cytoplasm, occasional foci of liquefaction necrosis, and proliferation of mononuclear cells (Fig. 5). In the hearts of animals that survived up to 48 hours, necrosis was much more marked and the proliferation of mononuclear cells more pronounced. Dehydrogenase activity in damaged fibers was either absent or markedly reduced (Fig. 6).

The Influence of Adrenalectomy

No significant decrease in dehydrogenase activity could be detected in the various organs, including the hearts of rats sacrificed 4 days following bilateral adrenalectomy, as compared with sham-operated and with unoperated normal control rats.

Comment

Seligman and his co-workers[8,9] were the first to draw attention to the staining pattern of succinic dehydrogenase in mammalian organs. They found that in various species, including the rat, mouse, dog, rabbit, guinea-pig, and hamster, the heart, kidney, and liver contained the larger amounts of stainable enzymatic activity. The myocardium of the human heart likewise revealed large amounts of stainable succinic dehydrogenase.

The abundance of this enzyme in the heart muscle of all species so

far examined indicates its fundamental importance for the metabolic process which takes place during cardiac contraction. It is now recognized that succinic dehydrogenase is one of the most important enzymes in biologic oxidation. By fractionation of the heart muscle into three components, (1) residue containing myofibril fragments, connective tissue, and nuclei, (2) sarcosomes, and (3) sarcoplasm, it was found that the highest specific activity of respiratory enzymes including succinic dehydrogenase is located in the sarcosome fraction.[10] Since sarcosomes (mitochondria) are arranged along the myofibrils in a longitudinal fashion, it is reasonable to assume that the granular deposits of reduced tetrazolium occur in the vicinity of these structures.

The loss of stainable dehydrogenase in muscle fibers undergoing necrobiotic changes due to anoxia is very striking. Diminution of enzymatic activity apparently exceeds changes which can be demonstrated by usual routine staining. Similarly, in experimentally induced renal necrosis, impending cell death is accompanied by loss of dehydrogenase activity.[1] In contrast to the kidney, however, there is no reappearance of dehydrogenase in necrotic areas in the heart since there is no regeneration of muscle cells. Reduction in dehydrogenase activity occurs also in muscle fibers that are slowly degenerating due to chronic anoxia.

The time relationship between the changes in dehydrogenase activity and gross or microscopic alterations in the myocardium following the exclusion of the blood supply could be studied in an experimental animal, since ligation of branches of coronary vessels can be performed without great difficulty in the dog.[11] For practical reasons, experiments of this type could not be carried out. However, a number of experimental procedures are known which will induce myocardial necrosis in smaller animals. The procedure chosen was the induction of acute myocardial damage by two chemicals, fluoracetate[5] and plasmocid.[6] The latter substance led to more consistent results in our hands. Plasmocid induced a varying, but often marked degree of myocardial damage within 24 hours. In damaged muscle fibers, the reduction of enzymatic activity appeared similar to that in the human heart following the occlusion of coronary vessels. In these experiments, as in those on renal necrosis, alterations in enzymatic activity were more widespread than the changes which could be detected with routine stains.

In contrast, no changes in dehydrogenase activity were found in the heart muscle following adrenalectomy. The different results obtained by us may well be explained by the different method of pre-

paring the sections for enzymatic staining. With thick sections incubated in a mixture without added activators, as used by Bourne and Malaty,[3] only inconsistent staining results were obtained. If, however, activators were added to the incubation mixture and thin sections prepared, no difference could be detected between preparations from adrenalectomized and control rats.

The resistance of histochemically demonstrable dehydrogenase to post-mortem changes is quite striking. In order to simulate conditions prevailing in human necropsy material, a portion of the heart was removed from 3 rabbits immediately after they were sacrificed. The thorax was then closed by sutures and the cadavers left for 3 hours at room temperature and then up to 24 hours in the ice box. No significant changes in the staining pattern could be seen in sections prepared from the heart immediately after death and in those prepared after termination of the experiment.

The fact that dehydrogenase activity remains active at least up to 15 hours after death clearly indicates that tetrazolium salts are not reduced by living tissues only, as was originally assumed. Indeed, under favorable conditions, even tissue extracts can reduce tetrazolium salts. Nevertheless, differences in tissue viability may be detected by their use.[12] It must be assumed that early post-mortem changes have considerably less damaging influence upon the succinic dehydrogenase system than the changes which occur during cellular death intravitam, regardless of the agent responsible for the cellular destruction. These observations clearly indicate that post-mortem changes and necrosis occurring during life cannot be discriminated by simple procedures.

From a practical point of view it is important to realize that post-mortem changes do not interfere with the staining reaction, at least for several hours. This enables one to prepare sections for testing succinic dehydrogenase activity not only from experimental material but also from that obtained by necropsy. Such preparations may be of help in evaluating the degree and distribution of myocardial damage.

Summary

The distribution of succinic dehydrogenase activity was studied in thin frozen sections of the human heart and of the heart of the rat under various experimental conditions. Only myocardial cells showed evidence of enzymatic activity. In post-mortem material, satisfactory staining could be obtained as long as 15 hours after death. In areas of myocardial necrosis following the occlusion of coronary vessels, de-

hydrogenase activity disappeared rapidly. Reduction of enzymatic activity was noticed as early as 2 hours after the onset of acute symptoms and extended to muscle fibers which showed no significant changes with routine staining techniques.

The administration of plasmocid and fluoracetate induced focal myocardial necrosis in the rat heart. In necrotic areas dehydrogenase activity was likewise reduced. In contrast, bilateral adrenalectomy had no influence upon dehydrogenase activity in the heart of the rat.

The use of the histochemical staining reaction for succinic dehydrogenase is suggested for the evaluation of myocardial damage under experimental conditions and in necropsy material.

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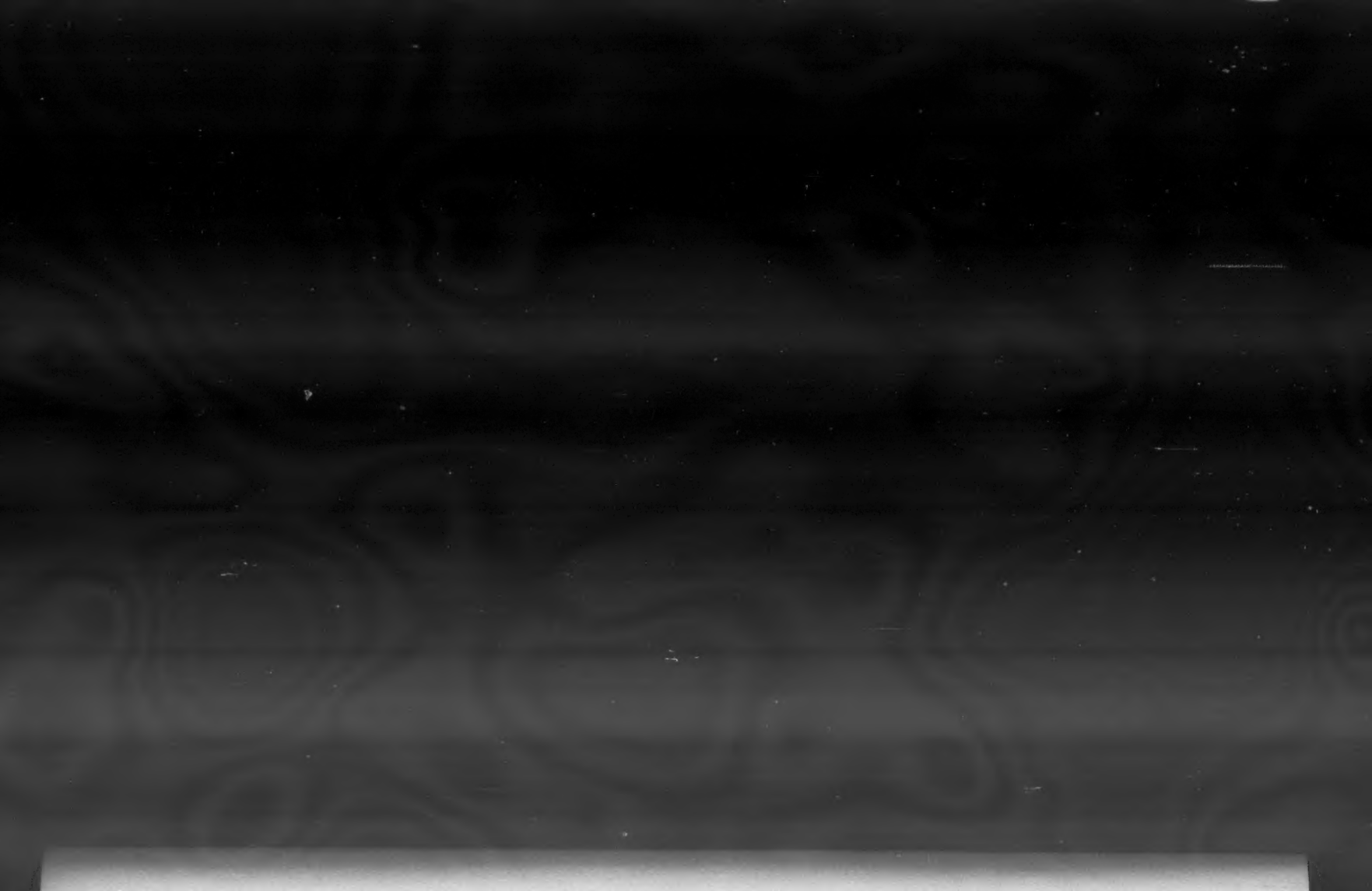
[*Illustrations follow*]

LEGENDS FOR FIGURES

All photomicrographs with the exception of Figure 5 are from fresh frozen sections cut at 10 to 15 μ and prepared for the demonstration of succinic dehydrogenase activity without counterstain.

FIG. 1. Human heart, left ventricle. Dehydrogenase activity is limited to myocardial cells. × 750.

FIG. 2. Section from the left ventricle of the heart of a 55-year-old man who died 2 hours after the onset of the typical clinical signs of acute coronary thrombosis. There is distinct diminution of the staining reaction in many myocardial cells. Sections of the adjacent areas stained with hematoxylin and eosin showed no evidence of acute necrosis. × 250.



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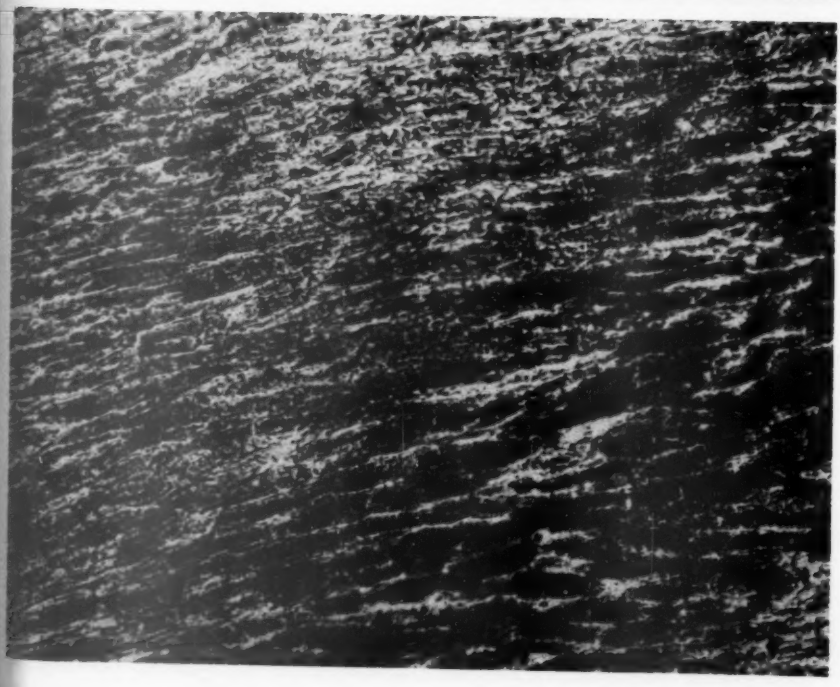

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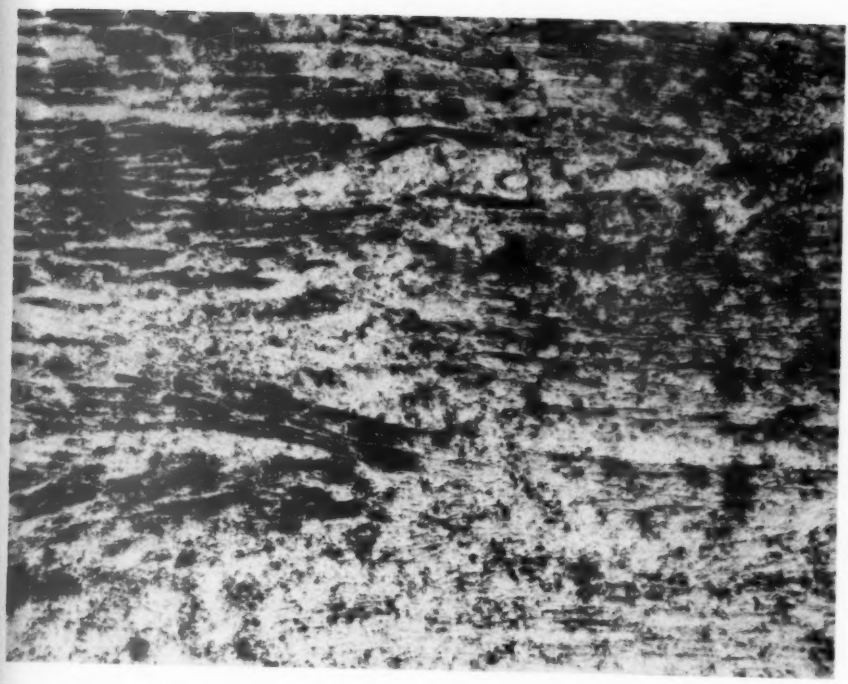
FIG. 3. Section from the left ventricle of a 49-year-old man, who succumbed 48 hours after the onset of the typical clinical signs of acute myocardial infarction. This section is from the edge of the grossly recognized infarct. There is complete loss of enzymatic activity in the necrotic muscle cells. × 125.

FIG. 4. Section through the subendocardial region of the septum of a 49-year-old woman with coronary heart disease who died of a cerebral hemorrhage. There is complete absence of staining in the fibrotic subendocardial area. Surviving muscle fibers show varying degrees of activity. × 125.



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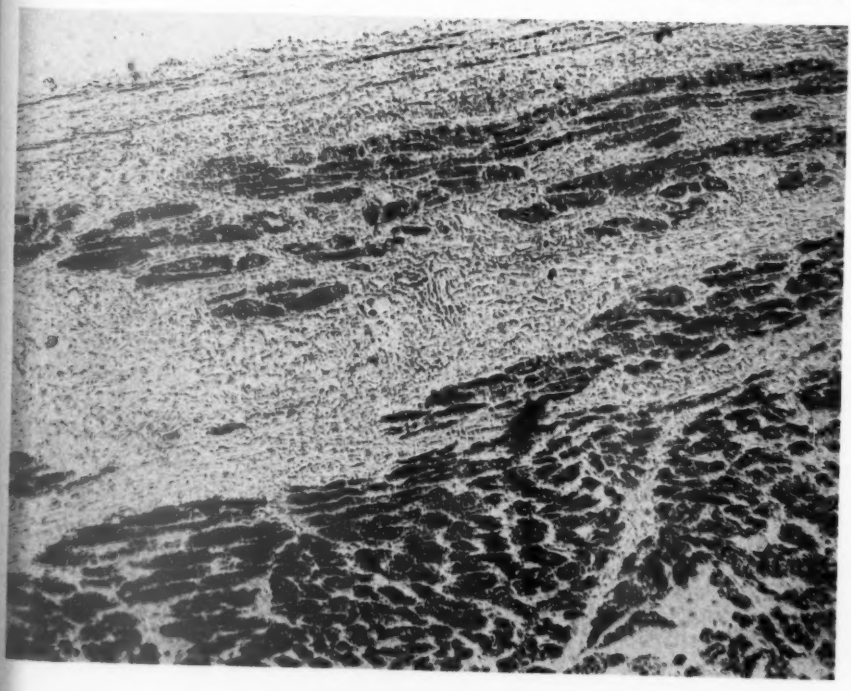

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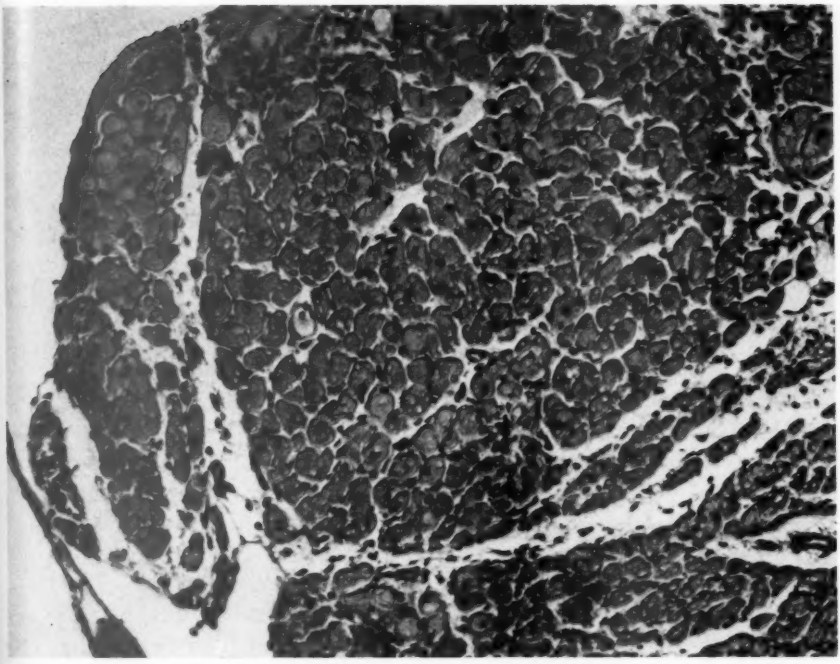
FIG. 5. Rat heart. The animal was sacrificed 22 hours after the second of two intraperitoneal injections of 6 mg. of plasmocid given 1 hour apart. There are focal areas of subendocardial myocardial damage and monocytic proliferation. Hematoxylin and eosin stain. × 250.

FIG. 6. Rat heart. Section was taken from an area of the heart adjacent to that shown in Figure 5. There is very extensive inactivation of the enzyme. × 250.



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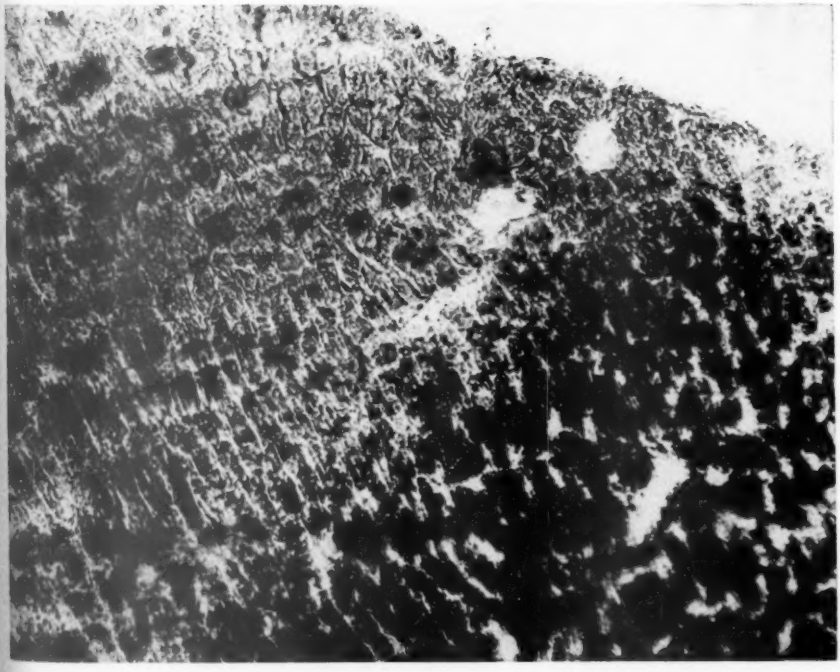

6

A CASE OF LEUKEMIC RETICULO-ENDOTHELIOSIS IN THE RAT

The Relationship Between This Entity and Naegeli's Monocytic Myelogenous Leukemia *

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The Fels Research Institute maintains three strains of rat leukemia by intraperitoneal transfer of 0.2 cc. either of whole blood or of a tissue mash of liver or spleen from a leukemic animal to rats less than 7 days of age. The chloroleukemia and acute lymphatic leukemia have already been reported[1-3]; a third leukemia, designated to date as Leukemia 149, constitutes the subject for this report.

This leukemia originated in a male rat, 29 months of age, the second generation offspring (F_2) of a female rat which, during lactation, received daily feedings by stomach tube of methylcholanthrene dissolved in olive oil. Whereas the F_1 mother may have imbibed methylcholanthrene in its mother's milk while nursing,[4] the F_2 animal in which the leukemia subsequently developed could not have come into direct contact with the carcinogen.

Inbreeding is avoided in the colony of Wistar rats maintained at the Fels Research Institute, so that, despite the fact that the leukemic animal developed from a brother-sister mating, it can be considered to have been *random bred*. The genetic and experimental factors which have influenced this animal preclude any definite statements as to whether the leukemia arose spontaneously or originated through some genetic mechanism related to methylcholanthrene.

Pathologic Findings

Splenomegaly, a spleen so large that the leukemic state can be diagnosed by abdominal palpation, characterized the gross findings. In the opened abdomen the spleen was seen to lie athwart and to obscure from view all viscera except for an occasional knuckle of small bowel. It was purple, distended, had obtuse edges, and accounted for one fifth of the animal's entire weight. The lymph nodes and thymus were enlarged to several times their normal volume, many measuring up to 1 cm. in cross-sectional diameter and 2 to 3 cm. in length. They were composed entirely of white, opalescent tissue which was soft and resilient. The bone marrow was dull pink, opalescent, and glutinous.

Extranodal tumor growth was found in the retroperitoneal fat sur-

* Received for publication, July 9, 1954.

rounding the kidney, extending caudad to cause obstruction at the bladder neck, cephalad to involve the porta hepatis, and anteriorly to infiltrate the mesenteric leaves, so that the mesentery resembled a thick white curtain.

The kidney contained numerous islands of soft, white, elevated tumor tissue. Invasion of kidney parenchyma occurred by way of the retropelvic fat rather than through the renal capsule.

The lungs and heart were invaded also.

Microscopic Description

On microscopic examination the heart was found to be invaded by contiguous growth of tumor cells from the thymus and thymic fat. The infiltration in the liver was confined to portal areas. That in the spleen was restricted generally to the red pulp, whereas in the lymph nodes a pannus-like sheet of cells replaced most architectural landmarks.

The leukemic cells, when seen in paraffin block section, were generally rounded or oval, and after the shrinkage caused by dehydration for paraffin embedding, they measured 6 to 10 μ in diameter. Their cytoplasm was pale pink and the nucleus usually appeared solid but occasionally was vacuolated. Variations in nuclear shape were pronounced, however, with oval, oblong, and reniform types interspersed among the predominant round forms. Mitotic figures were not abundant and there were no ring forms or polymorphonuclear granulocytes. In no instance did the cells in paraffin block sections contain granules.

The structure varied somewhat from organ to organ. In the lymph nodes there was relatively slight variation in size, shape, and staining characteristics of the cells; and foci of non-malignant, but hyperplastic reticulum cells were found occasionally amid the leukemic infiltrate. Normal follicular structure was never seen and, conversely, extracapsular extensions of tumor cells at the hilus were common. The tumor cells in the tissue were about the size of a small lymphocyte.

Splenic follicular architecture was replaced by a monotony of small cells, not too crowded, in the red pulp, not all of which could be identified as malignant cells. There was no reticulum cell hyperplasia and no extracapsular extension of the tumor.

The tumor in the lung was unusual in that striking morphologic variation occurred in different portions of the same lesion. Generally, there was considerable anaplasia and variation between cells with respect to size, shape, and staining characteristics. Indeed, the picture resembled that of a reticulum cell sarcoma as described by Gillman and Gillman[5] and seen, as well, in our colony (Figs. 1 and 2); and

often giant cells of the Sternberg type, mitotic figures, and other aberrant forms could be found.

The marrow, so viscid that when touched to the slide it would contract into a globule of pink tissue and defy the most careful and precise attempts to make thin imprints, showed displacement of most of the normal elements by a large agranular round cell found mostly in a degenerating form. The large azurophilic granules which characterized the cells of the peripheral blood in this leukemia (*vide infra*) were never uniformly demonstrated.

Peripheral Blood Smear

The blood smear made on November 5, 1951, indicated some fundamental morphologic deviations from the norm, which in the slide of November 21, 1951, appeared to have been intensified. On these occasions the count reached 58,300 and 57,000 cells per cmm. respectively, with the partition of each total as shown in Table I.

TABLE I

Differential Counts on Peripheral Blood

Cell type	Date of blood smear			
	11/5/51		11/21/51	
	Differential count*	Absolute count (based on initial count of 58,300)	Differential count*	Absolute count (based on initial count of 57,000)
	%		%	
Mononuclear cells with granules	44	25,000	47.3	27,000
(Those with lymphocyte-like nucleus)	(25)	(14,500)	(22.3)	(12,700)
(Those with blast-like nucleus)	(19)	(11,000)	(25)	(14,300)
Mononuclear cells without granules	13.6	8,200	17	9,700
(Those with lymphocyte-like nucleus)	(7.3)	(4,500)	(7)	(4,000)
(Those with blast-like nucleus)	(6.3)	(3,700)	(10)	(5,700)
Blasts	1.5	900	1.3	700
Small lymphocytes	19.5	11,300	6.8	3,900
Large lymphocytes	0.3	200	2.5	1,400
Monocytes	0.3	200	0.8	400
Segmented cells	15.5	8,700	19.0	10,800
Staff cells	4.8	2,800	5.0	2,800
Metamyelocytes	0.8	500	0.5	200

* Differential counts based on counts of 400 cells, and calculated to nearest tenth of a per cent.

In doing differential counts, the classical designation of cells was amended in part, because the atypical forms could not justifiably be categorized without overlooking pertinent atypical features. To insert these forms into familiar groups would obscure rather than emphasize the atypicality. Therefore, on a purely descriptive basis, two new categories were established and these, as well as the cells which we called blast cells in this leukemia, will now be defined.

Large mononuclear forms without granules are cells from 14 to 18 μ in diameter with pale blue or neutrophilic cytoplasm which may be clear or which may contain numerous small vacuoles giving a ground-glass appearance. Such cells have been identified in man with certain infectious processes. The nuclear chromatin may be arranged in thick blocks (Fig. 3), or else in finer strands (Fig. 4), sometimes similar to that of a blast cell. This category, then, includes cells which cannot be sharply defined as either large lymphocytes or large monocytes, but which often combine features of the two types, i.e., lymphocytic nuclei with foamy or ground-glass cytoplasm, or else a finely reticulated nucleoplasm as found in monocytes but of spherical form, and surrounded by the clear cytoplasm which is ordinarily identified with that of lymphocytes. Figures 3 and 4 represent two monocytoid cells, the first with definite pachychromatin in the nucleus, the second with younger nucleoplasm.

Mononuclear cells with granules are similar to the mononuclear cells without granules, except that they contain cytoplasmic azurophilic granules up to 2 μ in diameter in certain instances (Figs. 5, 6, and 7). These granules are discoid and fail to stain when subjected to Feulgen, Hotchkiss-McManus, methyl green-pyronin, or benzidine reactions. At times they are so large that they resemble cytoplasmic inclusions (Figs. 5, 7, and 8). The nuclei in these cells range from those which cannot be differentiated from the pachychromatin of a lymphocyte (Fig. 6) to that which resembles a blast nucleus (Fig. 5). The cytoplasm, however, is usually sky blue and clear. Some of these cells contain large "digestion" vacuoles which normally are empty (Fig. 9), or numerous vacuolar spaces surrounding the large azurophilic bodies (Fig. 8).

The *blast cells* have nuclei resembling those in the myeloblast described by Naegeli (or Ferrata's hemocytoblast), but the scanty cytoplasm is generally paler than that in the cells which they described and nucleoli are less common amid the leptochromatin. Some of them have fine azurophilic granules scattered throughout the cytoplasm, which would tend to identify them with the myeloblast of Ferrata (Naegeli's promyelocyte). A cell similar to a micromyeloblast is seen in Figure 10. This cell contains granules which are smaller than the ones described, more rounded and darker than the cherry red of the granules found in the *mononuclear cells with granules*.

Table I compares the absolute counts on the 5th and 21st of November, 1951, using these arbitrary designations.

These data show that, despite the leukemic state, the number of mature cells falls within the normal range for the rat, except that

there is a reversal of the lymphocyte/granulocyte ratio from the normal 3/1 in the rat to about 1/3.

Although the absolute differential counts of Nov. 5 and Nov. 21, 1951, were quite similar, in the latter count the large mononuclear cells with granules tended to have younger, more blast-like nuclei than did similar cells on the slide of Nov. 5. The granules also had become larger and some resembled cytoplasmic inclusions (Fig. 7).

Comment

Although the original leukemia had been diagnosed as a form of lymphatic leukemia, the ultimate form of the disease as delineated in the transferred animal falls into the group which embraces the monocytic leukemias, the leukemic reticulo-endothelioses.

The tendency of the leukemic cells to develop more blast-like nuclei is seen in the blood smear of Nov. 21, wherein there were almost half again as many cells with blast-like nuclei as in the blood smear of Nov. 5 (Table I). This tendency to form blast-like nuclei was responsible for the diagnosis of *monocytoid myelogenous leukemia* (after Naegeli) as made by numerous hematologists, in both the clinical and experimental fields, to whom we submitted blood smears. Because many of the cells have indented nuclei, sometimes kidney shaped, the agnomen *monocytoid* is acceptable but there is no reason to call this a myelogenous leukemia, since the cells are neither, as Naegeli believed,[6] derived from myeloblasts, nor do they bear direct relationship to any other normal myelogenous cell. Although the ultimate cell type can be related clearly by means of intermediate forms to the lymphocyte, it would be improper to classify the ultimate form as a lymphocytic leukemia because at this stage no morphologic resemblance to lymphocytes can be adduced. We would, therefore, prefer a name such as *monocytoid blast-cell* leukemia, to identify this specific form of the disease, which we believe properly belongs to the leukemic reticulo-endothelioses.

In man the diagnosis of monocytoid myelogenous leukemia (after Naegeli) implies that all the leukemic cells derive from myeloblasts, in contradistinction to Schilling's monocytic leukemia in which the leukemic cells are supposed to derive entirely from the extramedullary reticulo-endothelial system.[7] That the two types of leukemia classically have different morphologic appearances is unquestioned, Schilling's being typified by cells similar to these seen in Figures 3 and 4, whereas the cells in Figures 5 and 10 resemble more those of the Naegeli type. This case then, similar to what Naegeli called monocytoid myelogenous leukemia, supports Pappenheim's view that in

leukemia, myeloblast-like cells can derive from lymphatic cells of the extramedullary reticulo-endothelial system,[8,9] and Figure 11 shows that leukemic cells can even take on some of the morphologic characteristics of the nucleated red cell series. This does not prove, as Pappenheim suggested in his earlier writings, that normal hematopoiesis follows similar lines.

One of the historical reasons for the rigid separation of the Schilling and Naegeli types of monocytic leukemia is the fact that the myeloblast was originally described by Naegeli in normal marrow, but could not be found in the normal lymphatic tissue, even though Naegeli admitted that Jaffa-Fränkel saw them in abnormal lymphatic tissue.[10]

If the myeloblast was truly a specific marrow cell, it was reasoned that any leukemic cell resembling a myeloblast should also originate in marrow, thus the term "myelogenous" was used to identify this type of leukemia. The fact that under normal conditions the myeloblast is seen only in marrow should not influence the classification of leukemias because the leukemic cell is not the progenitor of a normal line of cells but rather the end result of an abnormal anaplasia. The leukemic cell ought not be named after a normal myelogenous component.

The so-called hiatus of Naegeli (Fig. 12) may also be responsible for the tendency to call leukemias of this type "myelogenous" or "myeloid," and may well be the basis for the oft heard axiom: the type of leukemic cell can often be determined from the company that the leukemic cell keeps. About the hiatus of Naegeli, Monasterio and Gigli,[11] studying leukemia in man, properly concluded: "After these considerations the so-called *'hiatus leukaemicus'* which according to Naegeli is one of the fundamental characteristics of the blood picture of acute leukaemia, cannot be considered as a *'hiatus,'* as the few granulocytes present in acute leukaemia do not derive, and cannot derive, from such cells, but instead from the scarse [*sic*] granuloblasts sometimes very difficult to discover in the great mass of atypical cells."

The azurophilic granules, sometimes so large that they resemble cytoplasmic inclusions, present an interesting and important clue to the derivation of the leukemic cell. Although the smaller granules are no different than the azurophilic granules of lymphocytes, the larger ones (Fig. 7), in so far as we have been able to determine, have been described only by Pappenheim and Ferrata[12] and are excellently illustrated in their Plate II. According to these authors the granules attain gigantic size often in the leukocytoid lymphocytes of the normal guinea-pig, and may be an intermediate stage of the development of

the Kurloff bodies of guinea-pig monocytes. In our study, these azurophilic granules and bodies found in the leukemic cells resembled chromatin material when stained with the Romanowski dyes, but failed to stain with benzidine, Hotchkiss-McManus, pyronin-methyl green, or Feulgen techniques, and gave a negative plasmal reaction. This would eliminate the possibility that the granules and larger bodies might be related to Auer bodies, virus particles, nuclear fragments and, according to the recent work of Nadel,[13] Kurloff bodies. Pappenheim and Ferrata, after entertaining the possibility that they might be related to the chromatin material of the nucleus, also suggested that they might be a secretion product of the cytoplasmic vacuoles with which they are often associated (Fig. 8). Identical granules and bodies were found in the histioleukemic cells in case 3 of Monasterio and Gigli.[11]

In summarizing, we would suggest that this leukemia in its ultimate form should be called a monocytoid blast-cell leukemia, an example of leukemic reticulo-endotheliosis. We believe that it demonstrates the evolution of Naegeli's type of monocytoid leukemia from an extramedullary source, and that the myeloblast-like cell is the end result of the abnormal process, not the cell from which the leukemic cells are derived. The possibility of a myeloblast-like cell developing in leukemic states from extramedullary reticulo-endothelial cells helps to explain certain of the cases of mixed leukemia which mystify the physician because of the metamorphosis of cells of lymphatic types to those of either monocytoid or even obviously granulocytic variety[8,14,15]; it also implies that Schilling's monocytic leukemia and Naegeli's monocytic leukemia are not two separate processes but two different stages of a single process.

Summary

The first case of leukemic reticulo-endotheliosis to be maintained in transfer in the rat is presented and its morphologic features described.

Characteristic of many of the leukemic cells is the presence of large azurophilic granules or bodies in the cytoplasm which serve to identify the leukemic cell with lymphocytic or monocytic forms, rather than with the myelogenous series.

Although many forms of the leukemic cell resemble myeloblasts, it is suggested that the so-called monocytoid myelogenous leukemia is not a disease originating specifically from marrow elements, as Naegeli supposed, but rather it is the result of an anaplastic process of the reticulo-endothelial system that might take place entirely in extramedullary loci.

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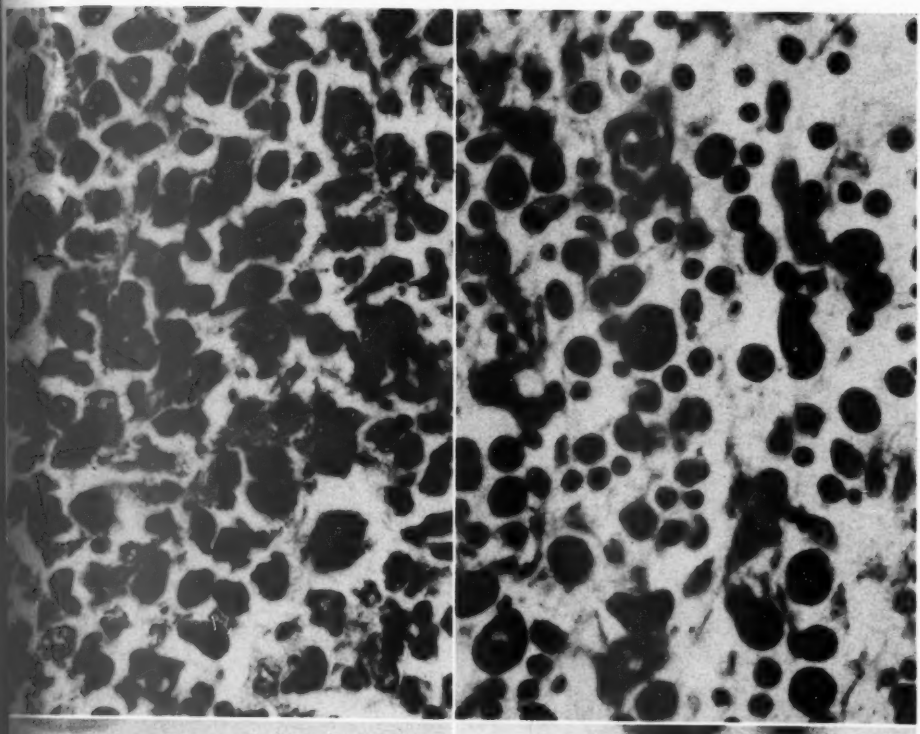
LEGENDS FOR FIGURES

FIG. 1. Section of tumor in lung showing infiltration of reticulum cell sarcoma type, characterized by cells with numerous cytoplasmic processes. × 600.

FIG. 2. Section of lung characterized by giant cells and extreme anaplasia. × 600.

FIG. 3. Monocytoid cell with lymphocyte-like nucleus. × 3,000.

FIG. 4. Cell similar to that shown in Figure 3 except more anaplastic. × 3,000.



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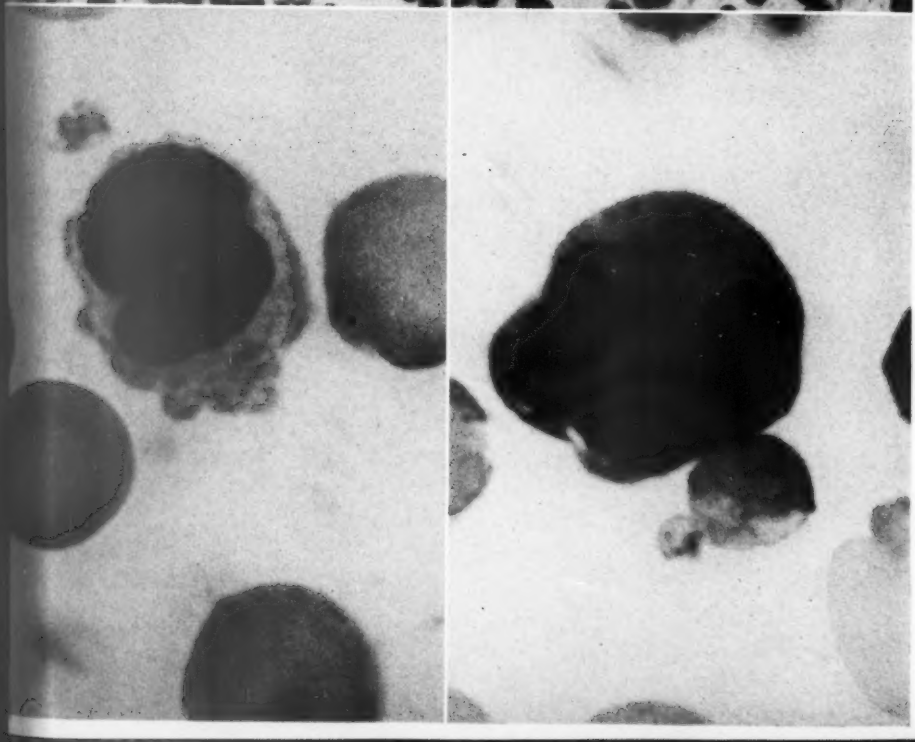

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FIG. 5. Large monocytoid cell with cytoplasmic granules and immature nucleus. × 3,000.

FIG. 6. A lymphocyte-like leukemic cell with azurophilic granules. × 3,000.

FIG. 7. Large azurophilic bodies in leukemic cells. × 3,000.

FIG. 8. Vacuolization of cytoplasm associated with the granules in leukemic cells. × 3,000.

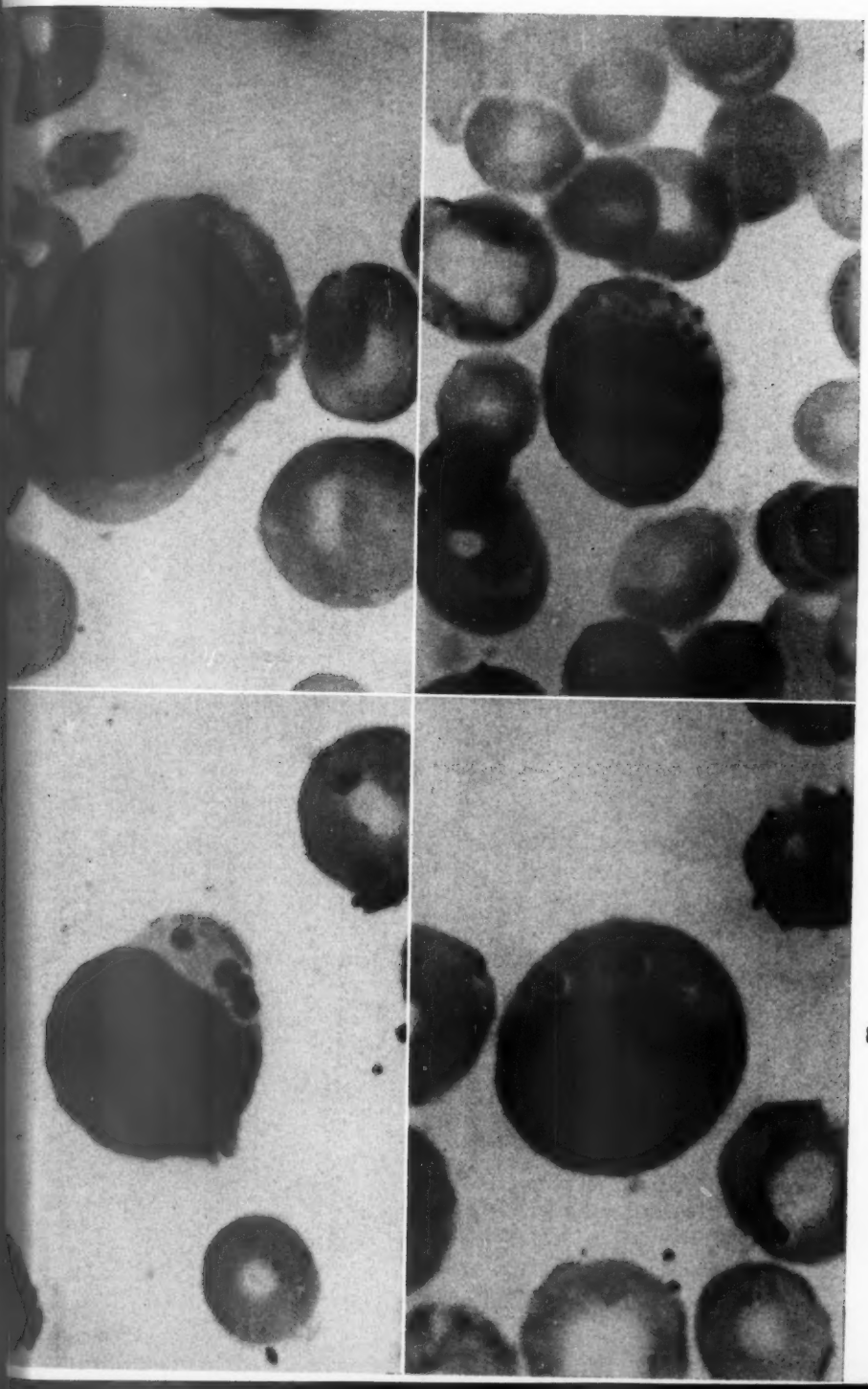

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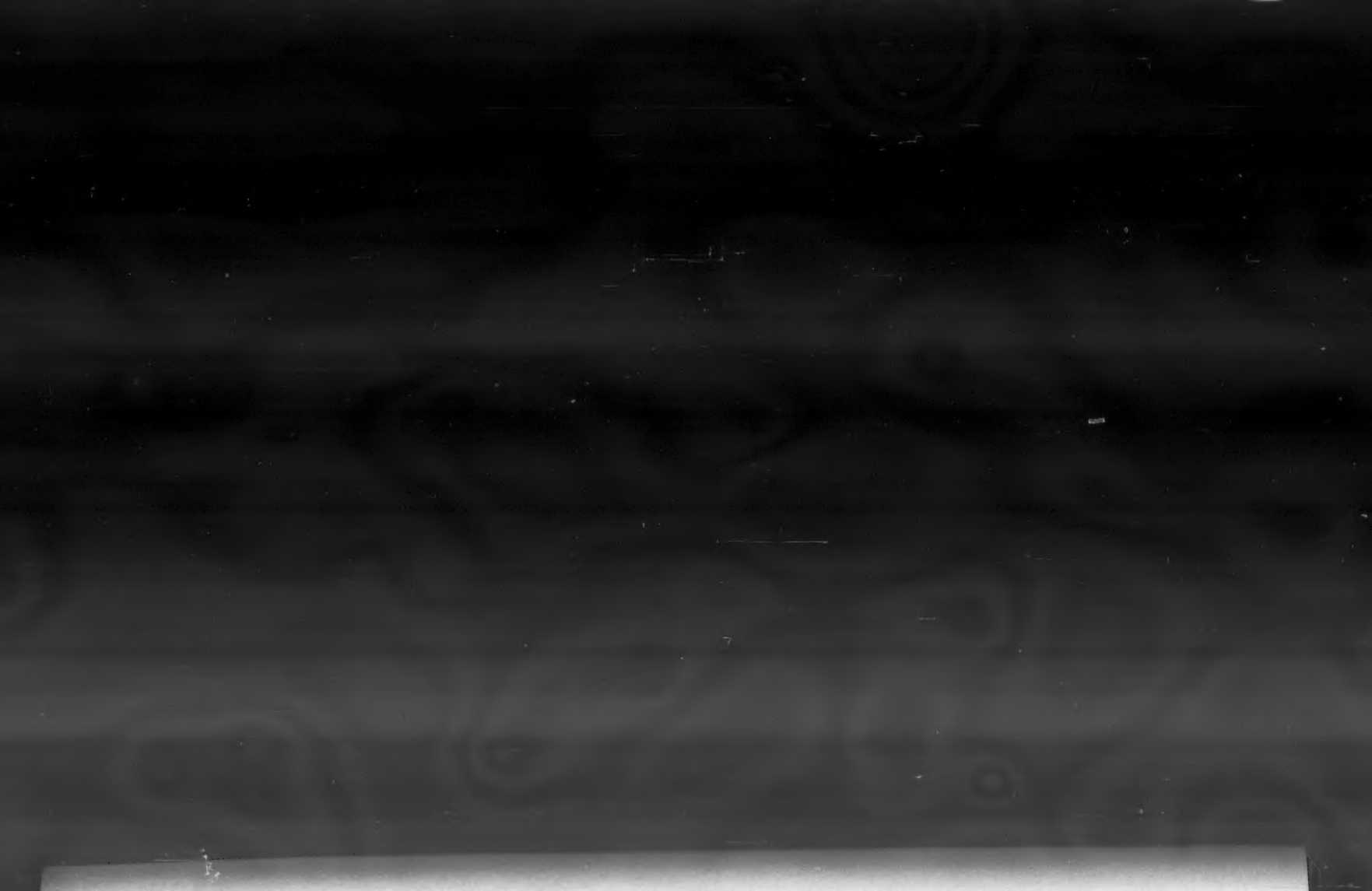
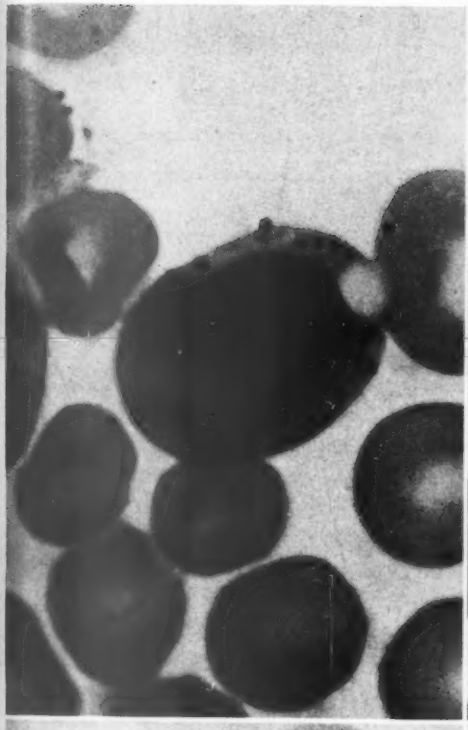
FIG. 9. Monocytoid cell containing a large cytoplasmic vacuole. × 3,000.

FIG. 10. Micromyeloblastic type of cell. × 3,000.

FIG. 11. Abnormal type of nucleated red cell deriving from the leukemic cells. There is a small collection of azurophilic granules at one pole of the cell. × 3,000.

FIG. 12. Juxtaposition of monocytic blast cell and segmented cell with granular cytoplasm to demonstrate the hiatus leukaemicus of Naegeli. × 3,000.





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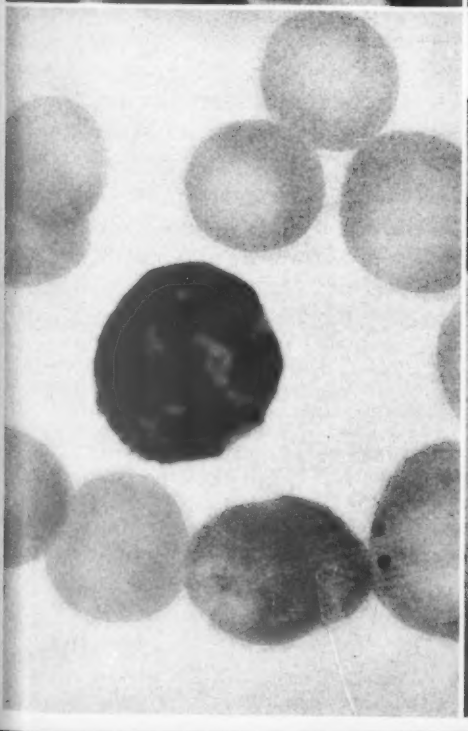

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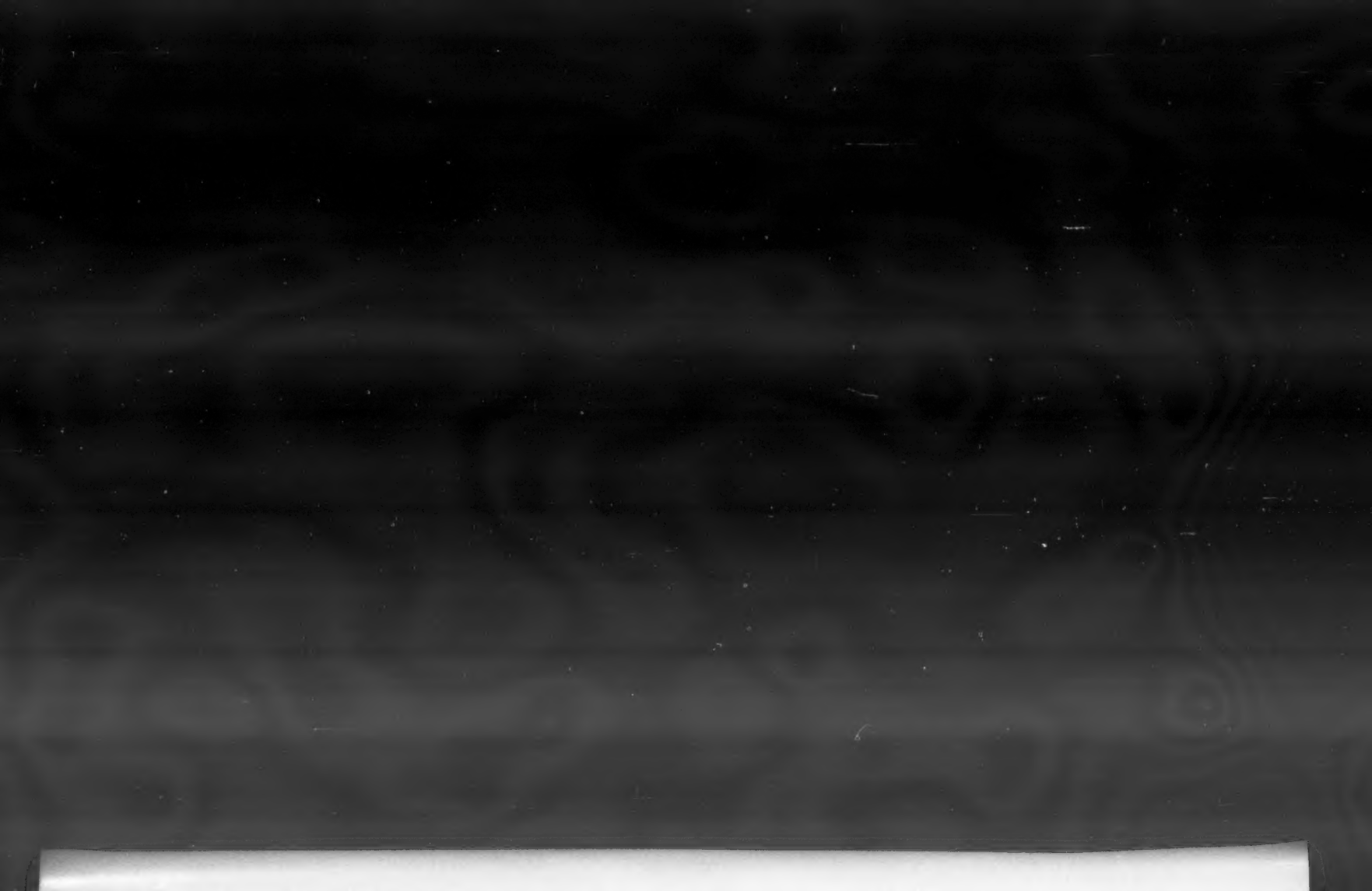
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